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ECBC-TR-512

DEVELOPMENT OF A PARTICLE COUNTING METHOD FOR ASSESSING THE BIOLOGICAL PROTECTION FACTOR OF RESPIRATORS

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November 2006

ABERDEEN PROVING GROUND, MD 21010-5424

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
XX-11-2006	Final	Nov 2003 - Nov 2005

4. TITLE AND SUBTITLE
Development of a Particle Counting Method for Assessing the Biological Protection Factor of Respirators

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER
20602384BPO

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES)
DIR, ECBC, ATTN: AMSRD-ECB-RT-PR, APG, MD 21010-5424.
Battelle Memorial Institute, 505 King Avenue, Columbus, OH 43201-2693.
Science Applications International Corporation, 3465A Box Hill Corporate Drive, Abingdon, MD 21009-1201

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-512

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This investigation developed and validated a highly sensitive method based on particle counting technology for assessing the protection afforded by chemical, biological, radiological, and nuclear respirators against biological threat agents. Inert monodisperse and polydisperse aerosols, representative in size to biological agents, were used to challenge a respirator fitted to a headform or worn by a human test participant. An aerosol spectrometer was used to measure aerosol penetration into the facepiece of the respirator. To demonstrate the method, monodisperse test aerosols and controlled mask leaks were used to generate an array of simulated protection factors (SPF) on the headform test apparatus. Human test participants were challenged with a polydisperse aerosol, which allowed for the measurement of several size specific protection factor (PF) values from a single test. Each test participant wore at least one expertly fitted respirator and several respirators with induced leaks in the peripheral seal to generate a wide range of PF measurements. A test to determine the effect of human generated background particles within the respirator was also conducted. In general, SPF and PF values were found to increase as the challenge aerosol particle size increased. Although subject generated in-mask background particles were found to limit the maximum measurable PF, the particle counting method sensitivity was sufficient to measure PF values up to 100,000.

15. SUBJECT TERMS
Protection factor; Mask leakage; Particle counting; Biological agent; Respirator; Aerosol penetration; Biological simulant

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Sandra J. Johnson
U	U	U	UL	47	19b. TELEPHONE NUMBER *(include area code)* (410) 436-2914

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PREFACE

The work described in this report was authorized under Project No. 20602384BP0, Research, Development, Testing and Evaluation. This work was started in November 2003 and completed in November 2005.

In conducting the research described in this report, the investigators adhered to Army Regulation 70-25, Research and Development – Use of Volunteers as Subjects of Research, dated 25 January 1990, as promulgated by the Office of the Surgeon General, Department of the Army. Approval for the use of human volunteers was granted by the Human Use Committee, U.S. Army Edgewood Chemical Biological Center, Protocol Log No. 0504S.

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Acknowledgments

The authors would like to extend a special thanks to all the test volunteers for their enthusiastic participation.

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DEVELOPMENT OF A PARTICLE COUNTING METHOD FOR ASSESSING THE BIOLOGICAL PROTECTION FACTOR OF RESPIRATORS

1. INTRODUCTION

Respiratory protection systems used for military and homeland defense applications must protect against a wide range of chemical, biological, radiological and nuclear (CBRN) hazards. The effectiveness of a CBRN respirator to protect the wearer against airborne hazards is quantified in terms of a protection factor (PF). The PF of a respirator is defined as the ratio of the challenge concentration to the concentration measured inside the respirator facepiece. Biological weapons pose a unique threat to military and civilian populations since they are usually invisible, odorless, exhibit latent effects, and are not easily detectable compared to conventional chemical warfare agents. Infectious biological agents such as anthrax, small pox, and tularemia are of particular concern since inhalation of a relatively small number of organisms can result in a lethal dose. For these and other reasons, the level of respiratory protection required for biological agents is in general at least an order of magnitude higher than that needed for chemical agents.

The standard aerosol-based test method used by the U.S. military, U.S. Department of Defense (1992), and the National Institute for Occupational Safety and Health (NIOSH) (2003) to quantify the protection level of CBRN respirators does not have sufficient sensitivity to measure PF levels needed for biological protection. The test method uses a polydisperse corn oil aerosol challenge with a mass median aerodynamic diameter (MMAD) of 0.4 to 0.6 micrometers (μm) that is intended to represent both vapor and aerosol chemical threat agents with respect to mask seal leakage. A light-scattering aerosol photometer is used to measure the challenge and in-mask concentration. Since the health effects of infectious biological agents are related to the total number of organisms inspired, particle counting methods offer a better means of determining the biological PF performance of respirators as opposed to photometer-based methods, where the PF measurement is indirectly related to the mass of the test challenge in terms of an exposure dose.

The purpose of this investigation was to develop and validate a highly sensitive method based on particle counting technology for assessing the protection afforded by CBRN respirators against biological threat agents. Inert non-toxic aerosols representative in size to biological agents were used as simulant test challenges. The study was conducted in two phases. In the first phase, the method was demonstrated using a test headform, monodisperse test aerosols, and controlled mask leaks to generate simulated protection factors (SPF). The second phase was conducted using human subjects, a polydisperse test aerosol, and induced mask leaks to produce a wide range of PF measurements. In both phases, a range of particle size challenges was assessed to determine the effect of particle size on in-mask penetration.

2. METHODS

2.1 Phase 1–Headform Simulated Protection Factor Study

2.1.1 Headform Simulated Protection Factor Test System

The headform test system (Figure 1) consisted of an aerosol generation system, an exposure chamber, a metal headform, a modified respirator, a breathing pump, and an aerosol sampling system. A schematic of the test system, illustrated in Figure 2, provides additional details. A condensation monodisperse aerosol generator (CMAG) (Model 3475, TSI, MN) was used to generate high concentrations of monodisperse aerosol challenges in the size range of 0.6 to 5.0 μm to simulate biological threat agents. Poly-alpha olefin (Emery 3004^{TM}) was used as the test challenge. Emery 3004^{TM} oil, which is in the paraffin hydrocarbon chemical family, is a colorless, odorless liquid used in industry as a synthetic lubricant. It was chosen as the test challenge since it has been approved by the U.S. Army Surgeon General as a nontoxic replacement for dioctyl phthalate (DOP) in respirator and filter leakage test systems.

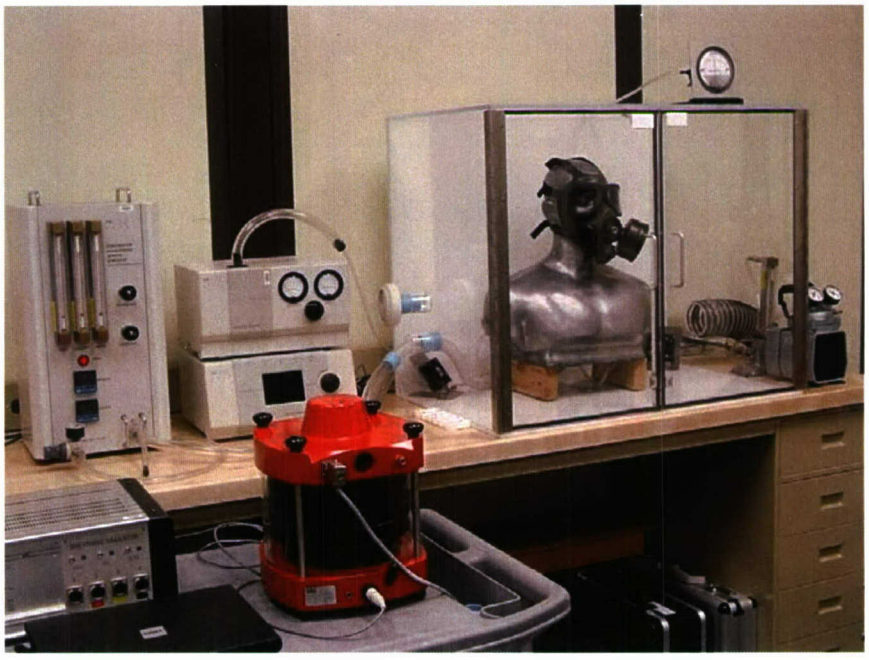

Figure 1. Headform Test System

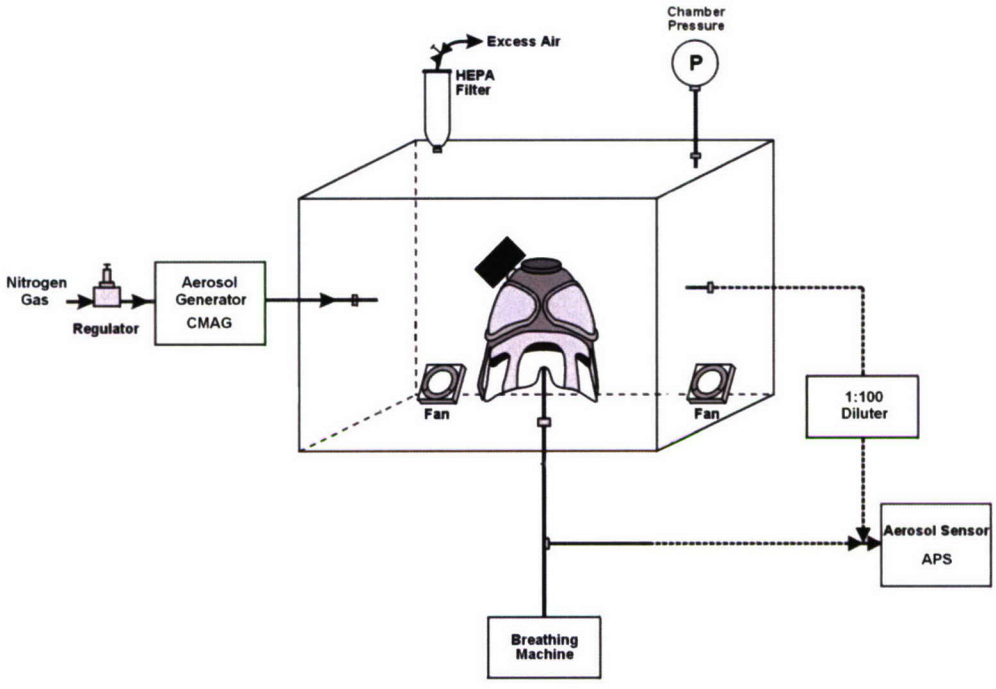

Figure 2. Headform Test System Schematic

The CMAG operates on the Sinclair-LaMer principle and involves condensing a vapor onto a condensation nucleus to form a monodisperse aerosol challenge. Sodium chloride is used as the condensation nucleus and oil with low volatility such as Emery 3004^{TM} or DOP is used for the condensate. Using nitrogen as the carrier gas, the salt nuclei travel thru a thermostatically controlled saturator chamber where vaporized oil condenses on the nuclei to produce a test challenge within the desired size range. Particle size and concentration are adjusted by controlling the saturator temperature and the proportion of total flow passing through the saturator unit.

The CMAG continually delivered aerosol-laden nitrogen gas to the exposure chamber. Within the chamber, mixing fans were used to ensure a steady well-mixed challenge was maintained. Excess challenge was vented into the room through a high efficiency particulate air (HEPA) filter located at the side of the chamber.

The exposure chamber contained the challenge atmosphere and housed the headform. The headform was equipped with a rubber bladder along the face periphery that was inflated with a laboratory pump to seal the respirator tightly to the headform. A military M40A1 CBRN respirator equipped with a C2A1 canister was mounted to the headform (Figure 3). A stainless steel bulkhead (i.e., pass-through fixture) located in the center of the mask voice transmitter was used to hold the leak sources. A series of thin, laser-drilled, stainless steel orifices (National Aperture, NH) were used to simulate direct leaks into the mask. The orifices could easily be inserted or removed from the mask bulkhead fitting during testing. The headform-respirator system was attached to a breathing pump (Computerized Breathing Simulator, FENZY, France). The breathing

pump was set to a minute volume of 25 L/min (1 liter tidal volume and 25 breaths/minute breathing rate). A sinusoidal breathing pattern was used during testing. The challenge atmosphere was pulled through the respirator canister and orifice during inhalation and exhaled through the respirator exhalation valve during exhalation.

Figure 3. Military M40A1 Respirator Modified with Bulkhead Fixture

An aerodynamic particle sizer (APS) (Model 3321, TSI, MN) and a 1:100 diluter (Model 3302A, TSI, MN) were used to measure the challenge and respirator (in-mask) aerosol number concentrations. The APS is a general-purpose particle counting spectrometer that measures the acceleration of particles within an accelerated aerosol stream to determine the particle aerodynamic diameter. Once the particle diameter is determined, each particle is counted and sorted in 1 of 32 bins (channels) ranging from 0.5 to 20 μm.

The chamber challenge concentration was measured with the APS diluter in-line using a probe that extended 10 centimeters (cm) inside the chamber (Figure 2). The in-mask aerosol concentration was measured with the APS from a probe located at the nose of the headform; no diluter was used. The aerosol sample and sheath air entered the APS through the sample line at 5 L/min. Tygon® tubing (Fisher Scientific, PA) was used for the challenge and respirator sample lines. The sample lines were kept the same length and as short as possible to minimize particle transport loss.

2.1.2 Test Procedure

The exposure chamber was filled with a stable aerosol challenge prior to orifice leakage testing. The CMAG saturator and reheater were set to 240 and 330°C, respectively. The test leak orifice was inserted into the bulkhead fitting and the fitting

sealed with a rubber stopper. The test chamber was closed and the chamber doorway sealed. The chamber sample line was then connected to the APS and diluter. The inflatable bladder between the respirator and headform was pressurized to approximately 3 pounds per square inch (psi) and the breathing pump was turned on. Once the CMAG reached the temperature set points, the nitrogen gas supply was opened and the flow was adjusted to achieve the desired particle size and concentration. After final adjustments were made and the concentration had reached steady state (typically after 10 to 15 minutes), sampling began. A description of the Phase 1 test system characterization is found in Appendix A.

An initial three-minute chamber sample was taken by the APS with the diluter inline. The diluter was then removed from the APS and a HEPA filter was connected to the sample port to purge the APS of any stray particles. The respirator (in-mask) sample line was opened and attached directly to the APS. A two-minute background sample was taken before the rubber stopper was removed from the orifice bulkhead fitting. After taking the background sample, the chamber was opened and the rubber stopper was promptly removed from the orifice. This was done as quickly as possible to prevent an excessive amount of particles from escaping the chamber. The headform was allowed to breathe for one minute before a three-minute in-mask sample was taken from the nose probe. Once completed, the APS was disconnected from the in-mask sample line, which was then sealed off, and attached to a HEPA filter for purging. The diluter and chamber sample line were attached to the APS and a final three-minute challenge sample was taken. Once the test was completed, the aerosol generator was shut down. The next aerosol size and orifice combination was prepared and the test was repeated.

2.1.3 Test Matrix

Five particle sizes and seven artificial leak conditions, including a sealed condition, were tested. The particle and orifice leak sizes are provided in Table 1. Particle sizes were selected to represent a variety of sizes within the limits of the CMAG (0.6-5.0 μm). The orifice sizes were chosen to obtain SPF values between 50 and 500,000. At least five trials of each particle and orifice size combination were completed for a total of 175 trials. The particle-orifice trials were randomized.

Table 1. Challenge Particle Orifice Sizes Tested

Particles (μm)	**Orifice (μm)**	
0.6	0 (sealed)	100
1.2	35	200
2.3	50	400
3.0	75	
5.0		

2.1.4 Data Analysis

Raw APS concentration data was automatically separated into 32 channels ranging from 0.5 to 20 μm. Only the channel closest to the selected particle size and the two channels above and below were used for SPF calculations (i.e., the concentrations of the five channels centered on the particle size of interest were summed together). The SPF was calculated as shown in Equation 1. The challenge concentrations taken before and after the in-mask concentration (C_{o1} and C_{o2}, respectively) were averaged. The SPF was calculated by dividing the average challenge concentration by the in-mask concentration (C_i) minus the background concentration (C_b):

$$SPF = \frac{(C_{o1} + C_{o2})}{2(C_i - C_b)} \quad (1)$$

The primary objective of Phase 1 was to establish a test method for estimating the biological protection factor performance of respirators using particle sizing and counting techniques. The aerosol generation and sampling procedures were refined while the study was in progress. As a result, the data from much of the SPF testing showed a great deal of variability. Therefore, a method was devised to exclude outlier data points. Only SPF results with initial and final challenge concentrations within 30% of each other and SPF results obtained with the exact test procedures described in section 2.1.2 were reported in the simulated PF final data set. All other data were removed. At least three trials from each particle-orifice test combination were used in the analysis.

All calculated SPF results were log transformed prior to analysis. The log SPF results were analyzed using analysis of variance (ANOVA) and regression techniques. ANOVA post-hoc multiple comparisons were completed using the Holm-Sidak method. Significance was accepted at the $p < 0.05$ level. Results are presented as means ± standard deviation unless otherwise stated.

2.2 Phase 2–Human Protection Factor Study

2.2.1 Human Protection Factor Test System

The human subject PF test system (Figure 4) consisted of an aerosol generation system, an exhaust blower system, an exposure chamber, and an aerosol sampling system. A 6-jet Collison nebulizer (BGI Instruments, MA) was used to generate a polydisperse corn oil (Mazola, TN) aerosol challenge. Corn oil was used in lieu of Emery 3004™ due to its wider acceptance as a non-toxic fit test challenge. This aerosol generation method was chosen instead of the CMAG used in Phase 1 since it did not require the use of nitrogen gas and thus was more cost-effective and easier to administer. The generator continuously delivered aerosol-laden air to the exposure chamber to maintain a stable aerosol challenge.

The exposure chamber was fabricated in-house by modifying a nylon outdoor shower tent (Townsend, CA). The tent was modified with the addition of clear vinyl windows, sample ports, an exhaust port, and a particulate filter dilution air inlet. Also to completely enclose the tent, a plastic floor was sewn onto the bottom edges. The exposure chamber was approximately 1 x 1 x 2 meters in dimension, providing adequate room for the test subjects to perform the exercise activities.

Dilution air entered the chamber through a 23 x 41 cm particulate filter located in the back. A mixing fan within the chamber combined the dilution and aerosol-laden air ensuring a uniform challenge atmosphere. A 5.0 horse power (HP) shop vacuum (Ridgid, MO) and a variable autotransformer (Staco Energy Products, OH) made up the exhaust blower system. The exhaust blower system continuously removed the challenge aerosol, thereby creating a constant inward flow of dilution air. The challenge air was filtered within the shop vacuum by a HEPA filter before being exhausted into the room.

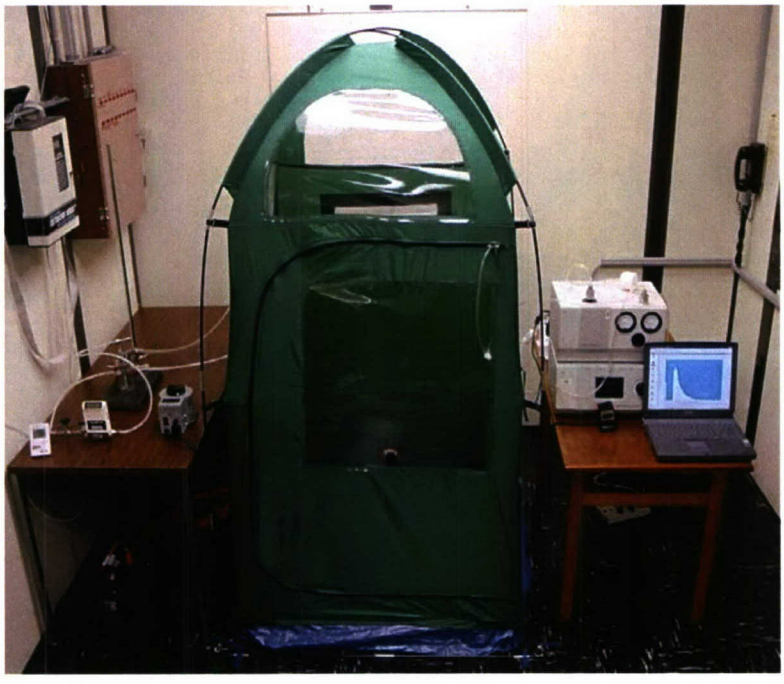

Figure 4. Protection Factor Test System

The same APS and 1:100 diluter used in the Phase 1 headform test were used to measure the challenge and respirator in-mask aerosol number concentrations. During normal operation, the APS pulls both the sample aerosol and sheath air through the sample line at a total flow rate of 5 L/min. For this test, a removable connector was attached directly to the APS sensor inlet port to permit sampling from within the respirator facepiece at a flow rate of 1 L/min. This was done to minimize potential inward leakage resulting from increased negative pressure caused by the higher sampling rate. The challenge sample was taken at the normal 5 L/min rate from a sample line that

extended approximately 10 cm into the top of the chamber. The sample line was located slightly above the test subject's head. The in-mask aerosol concentration was sampled directly from the mask drink tube (Figure 5). The mask's spring-loaded drink valve was replaced with a Teflon® quick-connect fitting to reduce sampling loss and facilitate connecting and disconnecting the sample line. The sample lines (Tygon® tubing), were kept the same length and as short as possible (approximately 2 meters) to minimize sampling loss while allowing enough length for the subjects to perform the exercises without restraint.

Figure 5. Commercial 3M FR-40 Respirator with APS Sample Line

2.2.2 Test Procedure

The exposure chamber was filled with a stable aerosol challenge prior to conducting respirator leakage testing. First, the exhaust blower and mixing fan were turned on. The exhaust blower airflow was regulated by adjusting the voltage output on the variable autotransformer. Next, a compressed air line preset at 20 psi was connected to the Collison nebulizer to begin the flow of corn oil aerosol to the chamber. A needle valve and a mass flow meter (Sierra Instruments, CA) were used to adjust the nebulizer flow to approximately 6 L/min. The nebulizer, mixing fan, and exhaust blower were run for at least 20 minutes to obtain a stable challenge atmosphere. The total particle count ranged from approximately 25,000 to 30,000 particles/cc. A description of the Phase 2 test system characterization is found in Appendix A.

After the exposure chamber was ready, the test subject donned a commercial 3M FR-40 CBRN respirator that was properly sized and fitted. A Portacount® (Model 8020, TSI, MN) quantitative fit tester was used to verify the seal of the respirator. The Portacount® is a portable quantitative fit testing (QNFT) device that uses a miniature condensation particle counter (CPC) to measure ambient aerosols inside

and outside of the respirator to determine the fit factor. The Portacount® has two modes of operation, allowing the user to either perform a quantitative fit test or use the device as a particle counter to monitor the aerosol concentration inside the respirator. The count mode was used to monitor the in-mask aerosol concentration while the subject breathed normally and the respirator fit was adjusted. Once the in-mask concentration reached zero, it was assumed the respirator was completely sealed. The Portacount® was disconnected and the drink tube was connected to the in-mask sample line. The subject then entered the chamber through the zippered entrance. The entrance was quickly opened and sealed to prevent challenge aerosol from escaping the test chamber.

Once the chamber was closed with the test subject inside, the aerosol challenge concentration was given an additional two minutes to stabilize before the APS measurements began. The APS and diluter were attached to the chamber (challenge) sample line and an initial two-minute sample was taken. Next, the diluter was removed from the APS and the in-mask sample line was attached directly to the 1.0 L/min APS sample inlet port. A few seconds were allowed for the APS to stabilize before the 5-exercise PF routine began. The exercise routine consisted of normal breathing (NB), turning head side to side (Head S2S), moving head up and down (Head U&D), bending over (Bend), and mimicking speech – lip-synching the "Rainbow Passage" (Fairbanks, 1960) (Mimic). Each exercise was performed for two minutes while a two-minute APS sample was taken. The exercises were always performed in the same order. Once in-mask samples at all five exercise conditions were taken, the in-mask sample line was disconnected, the diluter was reconnected to the APS, and the chamber sample line was attached. A final two-minute challenge sample was taken. After the trial was completed, the subject exited the chamber.

Each subject completed at least five different test conditions. At least one test condition consisted of a properly sealed respirator in an effort to determine each subject's best fit, or "baseline" condition. Once a baseline test ("sealed" condition) was completed, the remaining trials were conducted with various levels of respirator seal leakage. The leaks were chosen randomly and consisted of either a loose or misfit mask ("natural leak"), or a wire induced leak in the temporal seal of the respirator ("artificial leak"). This was done to obtain a wide range of PF measurements for analysis. The Portacount® was used as a tool to estimate the extent of the leak prior to the leakage trials. If the leak was too large or small, the respirator was adjusted accordingly.

2.2.3 Test Participants

Eleven subjects (6 males and 5 females) aged 30.5 ± 7.3 yrs (mean ± SD) were recruited for the study from the civilian workforce of the Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD. All participants were healthy and free of coronary risk factors as determined by completion of the OSHA respirator medical evaluation questionnaire. Each subject gave written informed consent prior to participation in the study, which had previously received institutional review board approval.

2.2.4 Data Analysis

The PF values of three particle sizes (0.6, 1.2, and 2.3 μm) were calculated for each subject exercise. Thus for each PF trial, 15 individual PF values were calculated (3 sizes x 5 exercises). Raw APS concentration data was automatically separated into 32 channels ranging from 0.5 to 20 μm. Three contiguous APS channels (one on, one above, and one below) were summed together to determine the concentration of the target particle size. The PF was calculated as shown in Equation 2. The challenge concentrations for each particle size taken at the beginning and end of each trial (C_{o1} and C_{o2}, respectively) were averaged. The PF was calculated by dividing the average challenge concentration by the in-mask concentration for each exercise (C_i):

$$PF = \frac{(C_{o1} + C_{o2})}{2(C_i)} \tag{2}$$

All calculated PF results were log transformed prior to analysis. Log PF results were analyzed using ANOVA and regression techniques. ANOVA post-hoc multiple comparisons were completed using the Holm-Sidak method. Significance was accepted at the $p < 0.05$ level. Results are presented as means ± standard deviation unless otherwise stated.

2.2.5 In-Mask Background Evaluation

After completing Phase 2, a test designed to determine the concentration of background particles (i.e., non-challenge particle levels) within the respirator was performed on 6 of the 11 test subjects. One trial was conducted per subject. The test consisted of eight different exercises; normal breathing (NB), deep breathing (DB), turning head side to side (Head S2S), moving head up and down (Head U&D), bending over (Bend), rotating jaw (Jaw R), speaking – reciting the "Rainbow Passage" (Speak), and mimicking speech – lip-synching the "Rainbow Passage" (Mimic). A powered air-purifying respirator (PAPR) hood was worn over the mask to provide a particle-free challenge while the subjects performed the exercises. The test was conducted in a lab room with ambient particle concentrations ranging from 4000 to 6000 particles per cubic centimeter (particles/cc). This test procedure was consistent with the procedure used in a QNFT study by Harrison and Liang (2005). In-mask particle background levels were measured with an APS. Since the challenge atmosphere was void of particulates, the background measurements only consisted of particles originating from within the respirator. Examples would include particles from exhaled breath and other potential sources such as the filter canister and inlet and outlet valves. A detailed description of the test is provided in Appendix B.

3. RESULTS

3.1 Phase 1–Headform Simulated Protection Factor Study

The log SPF results for each orifice-particle size test combination are displayed graphically in Figure 6. All log SPF results are presented in Table 2. The ANOVA analysis demonstrated that both particle size and orifice size were significant. Post-hoc comparisons showed the 5.0 μm particles to be significantly different from all other particles. The 3.0 and 1.2 μm particles were the only other sizes that were significantly different from each other. The five orifice sizes in the middle (35, 50, 75, 100, 200 μm) were statistically different, but within this orifice size range the adjacent orifice sizes were not statistically different. For example, the 50 μm orifice was not statistically different than the 75 μm orifice and the 75 μm orifice was not statistically different from the 100 μm orifice, but the 50 μm orifice was statistically different from the 100 μm orifice. The trends seen in Figure 6 were as expected; within a given particle size, the log SPF decreased with increasing orifice size (i.e., larger leaks equated to lower SPF values). Also, within a given orifice size, the log SPF values tended to increase as the challenge particle size increased.

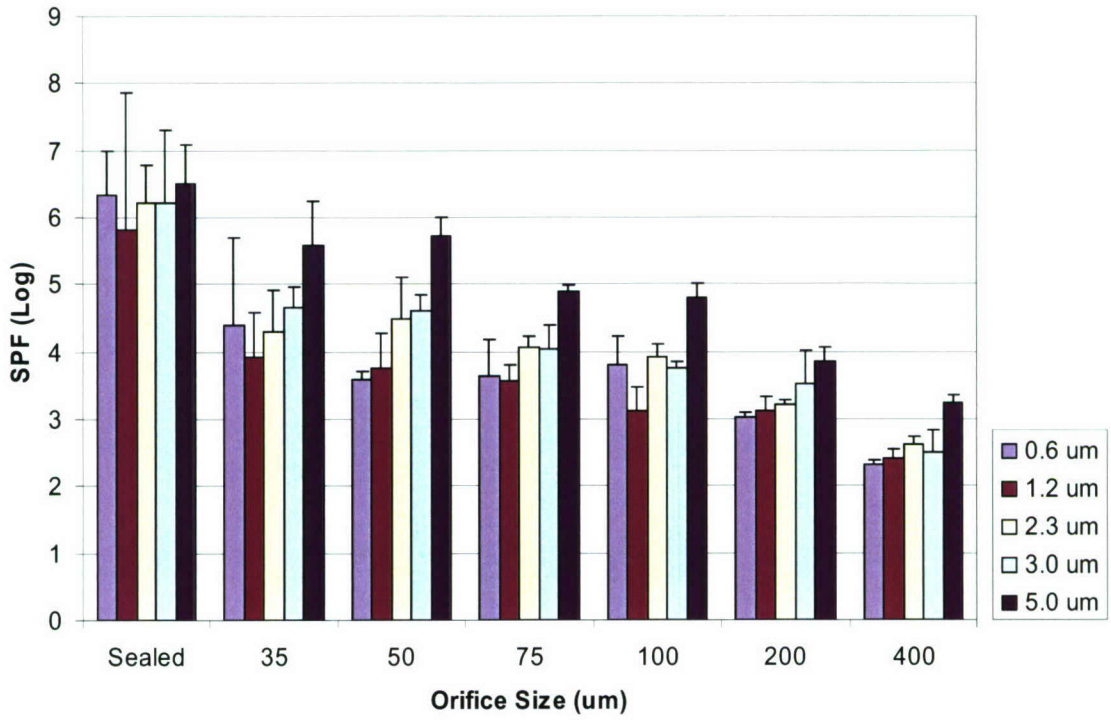

Figure 6. Log SPF Means at Each Particle and Orifice Size Combination

Table 2. Log SPF Results

Orifice Size (µm)	0.6 µm		1.2 µm		2.3 µm		3.0 µm		5.0 µm	
	Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev
Sealed (0)	6.3	0.7	5.8	2.0	6.2	0.6	6.2	1.1	6.5	0.6
35	4.4	1.3	3.9	0.7	4.3	0.6	4.7	0.3	5.6	0.7
50	3.6	0.1	3.8	0.5	4.5	0.6	4.6	0.2	5.7	0.3
75	3.6	0.5	3.6	0.2	4.1	0.2	4.0	0.3	4.9	0.1
100	3.8	0.4	3.1	0.3	3.9	0.2	3.8	0.1	4.8	0.2
200	3.0	0.1	3.1	0.2	3.2	0.1	3.5	0.5	3.9	0.2
400	2.3	0.1	2.4	0.2	2.6	0.1	2.5	0.3	3.2	0.1

The log SPF means of all particle sizes were plotted against the corresponding orifice size (Figure 7). The sealed condition log SPF values were often higher than the detectable limit. Since the "true" log SPF values were not known, the sealed log SPF means were not used in this analysis. Linear regression lines were also plotted within Figure 7. The linear regression line slopes, intercepts, and correlations for all five particle sizes are displayed in Table 3.

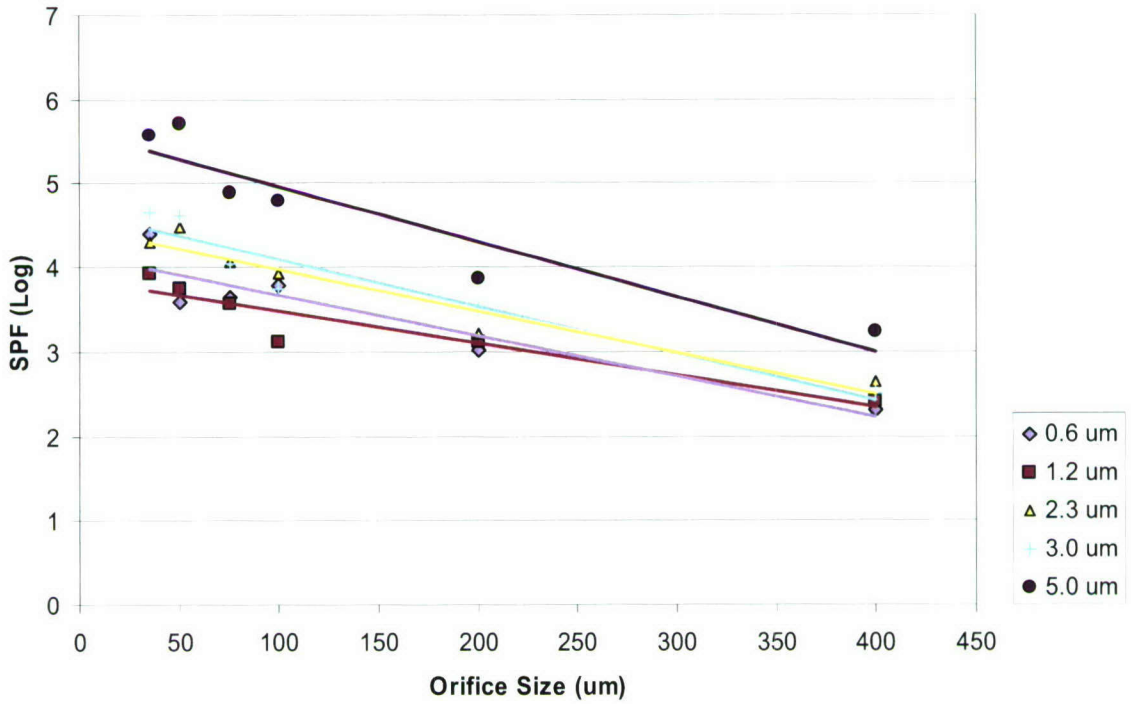

Figure 7. Log SPF Mean and Orifice Size Regression

Table 3. Log SPF Regression Coefficients

Particle Size (μm)	Slope	Intercept	R^2
0.6	-0.0048	4.14	0.86
1.2	-0.0037	3.85	0.89
2.3	-0.0049	4.47	0.94
3.0	-0.0055	4.64	0.92
5.0	-0.0065	5.62	0.88

A comparison of the regression slope and intercept coefficients from several of the particle sizes was completed using a method developed by McCuen (1993) to determine if the coefficients were statistically different. The 0.6 μm coefficients were compared to the 1.2, 2.3, and 5.0 μm coefficients. Since the 0.6 μm particle size approximated the MMAD of the corn oil aerosol challenge used in the standard military PF test protocol, it was used as the initial basis for comparison. The 5.0 μm particle size was selected because it was the only size significantly different from the 0.6 μm particle challenge using ANOVA. Also, the 1.2 and 2.3 μm particles were similar in size to those used for Phase 2. The slopes of all three particle sizes, 1.2, 2.3, and 5.0 μm, were not statistically different from the 0.6 μm particle slope. Also, the 1.2 and 2.3 μm intercepts were not statistically different from the 0.6 μm intercept. However, the 5.0 μm intercept was statistically different from the 0.6 μm intercept. Because the 1.2 μm particles were similar in size to the 0.6 μm particles and they were used in Phase 2, the 1.2 μm particles were also used as a basis of comparison. The 1.2 μm coefficients were compared to the 3.0 and 5.0 μm coefficients. The 3.0 and 5.0 μm particle sizes were selected since both were the only sizes that were statistically different from the 1.2 μm particle challenge in the ANOVA. Also, the 3.0 μm particle is close in size to the 2.3 μm particle used in Phase 2. The slopes of both the 3.0 and 5.0 μm particle sizes were not statistically different from the 1.2 μm slope, but the intercepts of both sizes were statistically different from the 1.2 μm intercept.

Although there were no statistical differences among the regression slopes, there were statistical differences among the intercepts. Thus, the intercepts were used to highlight trends within the particle sizes. Excluding the 0.6 μm intercept, the intercepts increased with increasing particle size. Since the slopes were virtually equal, an increase in the intercept value corresponded to an increase in log SPF. Furthermore, because the slopes are statistically equivalent, the intercept can be used to estimate the effect of particle size on the log SPF values. An increase in 0.6 μm particle size to 1.2 μm would cause the log SPF value to decrease by approximately 7%, and an increase in 0.6 μm particle size to 2.3 or 5.0 μm would cause the log SPF values to increase by approximately 8% or 36%, respectively. Also, an increase in 1.2 μm particle size to 3.0 or 5.0 μm would cause the log SPF values to increase by approximately 21% or 46%, respectively. It should be noted that with only 6 data points for each particle size, the degrees of freedom were relatively low. Thus, the percentages presented above are used only to illustrate trends.

3.2 Phase 2–Human Protection Factor Study

The human subject PF trial results are split into three sections. Section 3.2.1 describes the results of a respirator background shakedown investigation. Section 3.2.2 describes the baseline results. Section 3.2.3 describes the leakage results.

3.2.1 In-Mask Background Evaluation

The results of the in-mask background concentration for each particle size and exercise are displayed in Figure 8. The two-way ANOVA indicated that there were statistical differences among the eight exercise conditions and three particle sizes. The Holm-Sidak post-hoc test indicated speaking produced significantly more particles than all other exercises. The other exercises were not significantly different from each other. Significantly fewer 2.3 μm particles were detected than any other particle size. Although no statistically significant difference existed between the 1.2 and 0.6 μm background particles, there was generally a higher 0.6 μm particle concentration inside the respirator.

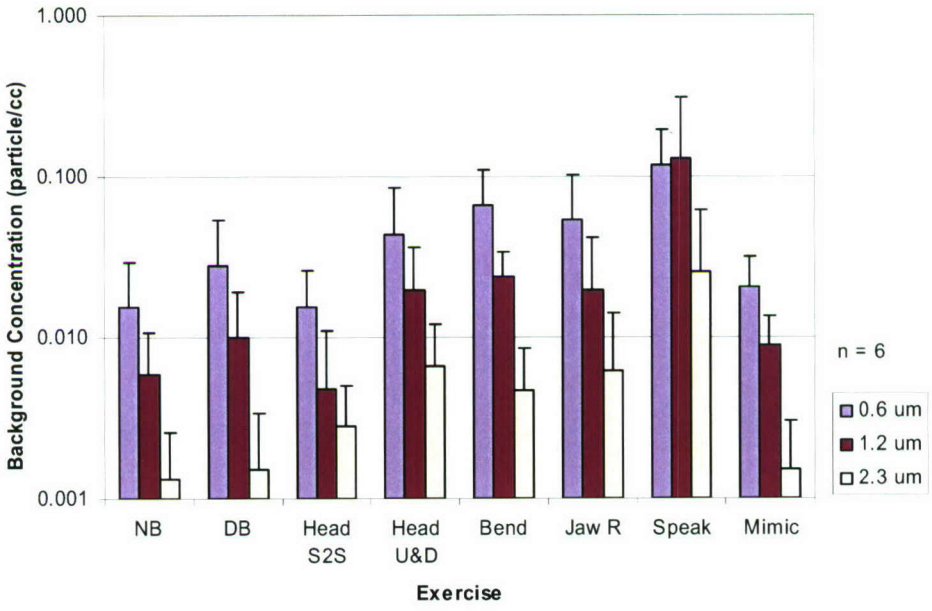

Figure 8. In-Mask Background Particle Concentration

Ideally, no background particles would be generated inside the respirator, and the in-mask concentration would only consist of challenge particles. In reality, there are background particles generated inside the mask. Since the particle measurement method could not distinguish between challenge and background particles, the measured in-mask concentration was the sum of the challenge (C_i) and background (C_b) particles. Thus, the measurable PF (PF_{pred}) can be predicted by Equation 3. The PF_{pred} divides the challenge concentration (C_o) by the measurable in-mask concentration ($C_i + C_b$):

$$PF_{pred} = \frac{(C_o)}{(C_i + C_b)} \quad (3)$$

Equation 3 was used to illustrate the effects of the background particles on the maximum measurable PF during each exercise. Assuming no respirator leakage (i.e., $C_i = 0$), the in-mask respirator atmosphere would only contain background particles. Since the particle measurement method can not distinguish between challenge and background particles, the method assumes all particles detected are challenge particles. As a result, the maximum measurable PF (PF_{max}) is calculated by dividing the challenge by the in-mask background concentration as shown in Equation 4:

$$PF_{max} = \frac{(C_o)}{(C_b)} \quad (4)$$

The average challenge concentration (C_o) measured for the 1.2 µm particle size during Phase 2 testing was 2,300 particles/cc. This value was divided by the average 1.2 µm in-mask background concentration (C_b) obtained during background testing to estimate the PF_{max} for each exercise. The estimated 1.2 µm PF_{max} results are shown in Table 4. With the exception of Speaking, the log PF_{max} was equal to or greater than 5 ($PF_{max} \geq 100{,}000$) for all exercises. The log PF_{max} for Speaking was only 4.3 ($PF_{max} = 20{,}000$).

Table 4. 1.2 µm Particle Maximum Measurable PF

Exercise	Max PF (Log)	Max PF
NB	5.6	400,000
DB	5.4	250,000
Head S2S	5.7	500,000
Head U&D	5.1	130,000
Bend	5.0	100,000
Jaw Rotate	5.1	130,000
Speak	4.3	20,000
Mimic	5.4	250,000

Although Table 4 displays the estimated PF_{max}, it does not illustrate the effect of the background particles on the PF as it approaches the measurable maximum. Assuming no background particles were generated within the respirator, and the in-mask concentration only consisted of challenge particles (i.e., $C_b = 0$), an optimum PF (PF_{opt}) would exist (Equation 5):

$$PF_{opt} = \frac{(C_o)}{(C_i)} \quad (5)$$

Using Equations 3 and 5, a method was developed to illustrate the effect of background particle concentration on the PF_{pred} as the PF_{opt} increases (i.e., as the mask leakage decreases).

The average background concentrations for 0.6, 1.2, and 2.3 μm particles taken from five of the eight exercises (NB, Head S2S, Head U&D, Bend, and Mimic) were used to represent the concentration of background particles inside the mask (C_b). The average challenge concentrations for the same size particles from the Phase 2 trials were used as the challenge concentration (C_o). Log PF_{opt} values were assumed to range from 3.0 to 6.0 (PF_{opt} ~ 1,000 to 1,000,000). These values were not calculated or measured. The log PF_{opt} values and average challenge concentrations were used with Equation 5 to back-calculate the in-mask challenge concentration (C_i). The calculated in-mask (C_i), average background (C_b), and average challenge (C_o) concentrations were input into Equation 3 to yield PF_{pred} values.

The effect of background particles on the log PF_{pred} is demonstrated in Figure 9. The dashed line denotes the ideal situation where the log PF_{pred} equals the log PF_{opt} (i.e., there are no in-mask background particles). The plots for the 0.6, 1.2, and 2.3 μm particles show that the background particles within the respirator cause the log PF_{pred} values to reach a maximum as the log PF_{opt} values approach 6 (PF = 1,000,000). For lower log PF_{opt} values (log PF_{opt} < 5), the log PF_{pred} and log PF_{opt} values are similar. However, as the log PF_{opt} value increases (log PF_{opt} > 5), there is a greater difference between the log PF_{opt} and the log PF_{pred} values. Hence, according to the data presented in Figure 9, it is predicted that the presence of background particles limits the ability to accurately measure log PF values greater than 5 (PF > 100,000). With the average background and challenge concentrations used for this analysis, it was estimated that the maximum log PF_{pred} values for all three particle sizes ranged from 5.1 to 5.4 (PF ~ 130,000 to 250,000). This method was prepared to provide an estimation of the effect of background particles on log PF_{pred} values, and thus an approximation of measurable PF. Increased challenge concentration or fewer background particles within the respirator would result in higher log PF_{pred} values.

3.2.2 Baseline PF Evaluation

The baseline condition for each subject was the highest PF value measured for any given PF trial. In all cases except two, the baseline trial consisted of a properly sized, fitted, and donned respirator (i.e., results from the "sealed" test condition). The 11 log PF baseline (PF_{base}) trial results, one for each subject, are shown in Figure 10. The log PF_{base} value is the average of the five individual Log baseline exercise PF (PF_{exe}) values. All 0.6 μm log PF_{base} values and most 1.2 and 2.3 μm log PF_{base} values were greater than 5 (PF_{base} > 100,000). The average Log baseline PF_{exe} values are shown in Figure 11. With the exception of the 1.2 and 2.3 μm log PF_{exe} values during the bending exercise, all baseline log PF_{exe} averages were greater than 5 (PF_{exe} >100,000). Since the log PF_{base} values were close to the detection limit of the method, no statistical analysis was done. The overall log PF_{base} results (i.e., the average log PF_{base} values of the 11 subjects) for 0.6, 1.2, and 2.3 μm particles were 5.5 ± 0.3 (PF ~ 320,000), 5.3 ± 0.4 (PF ~ 200,000), and 5.3 ± 0.4 (PF ~ 200,000), respectively.

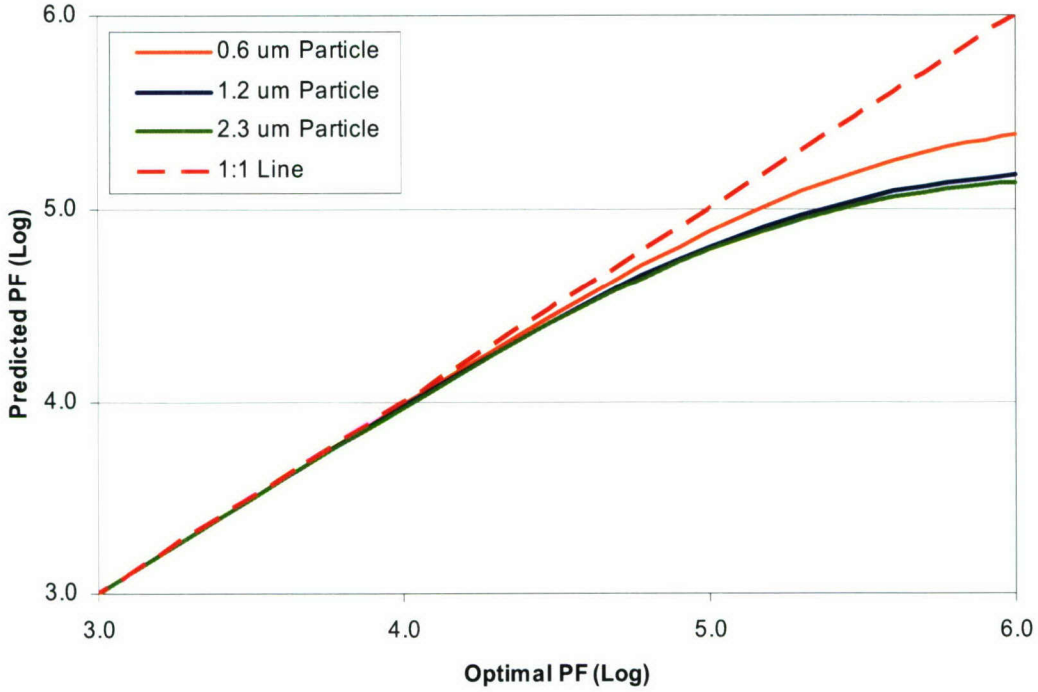

Figure 9. Predicted Measurable log PF

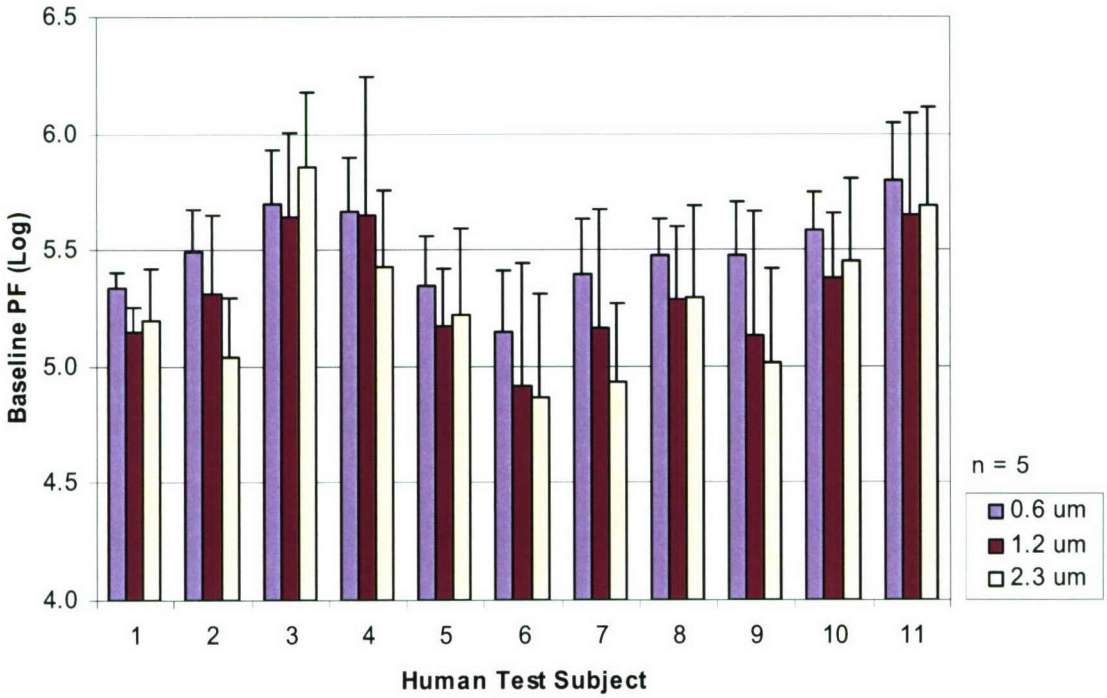

Figure 10. Mean log PF_{base} for Each Subject and Particle Size

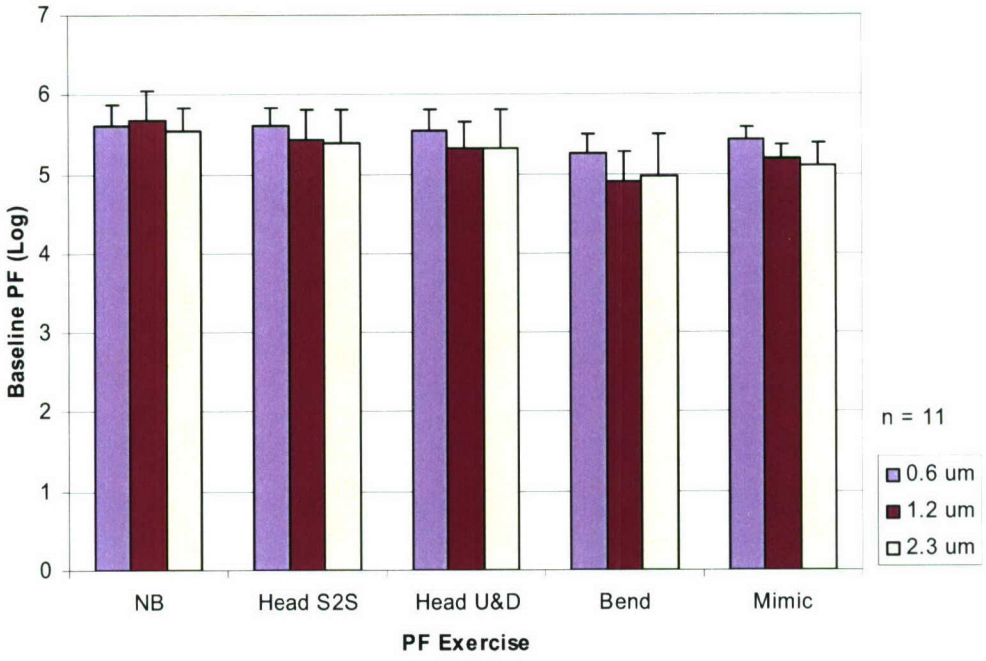

Figure 11. Mean Baseline log PF_{exc} for Each Particle Size

3.2.3 Leakage PF Evaluation

The 0.6 μm log PF results were separated into four ranges, 0-3 (1-1,000 PF), 3-4 (1,000-10,000 PF), 4-5 (10,000-100,000 PF), and >5 (>100,000 PF). The 0.6 μm particle size was used as the independent variable since it approximated the MMAD of the corn oil aerosol challenge used in the standard military PF test protocol. Each data set included a 0.6, 1.2, and 2.3 μm log PF_{exc} value. Within the selected ranges, the averages of the 0.6 μm log PF values and the corresponding 1.2 and 2.3 μm log PF values were plotted in Figure 12. For the first three ranges, the 0.6 μm log PF values were the lowest while the 2.3 μm log PF values were the highest. In the last range (log PF > 5), this trend disappears since the majority of the log PF values exceeded the upper measurement limit of the test method. This resulted in highly variable PF data. Thus, the data sets with a 0.6 μm log PF value above 5 were not used in the subsequent particle size analysis.

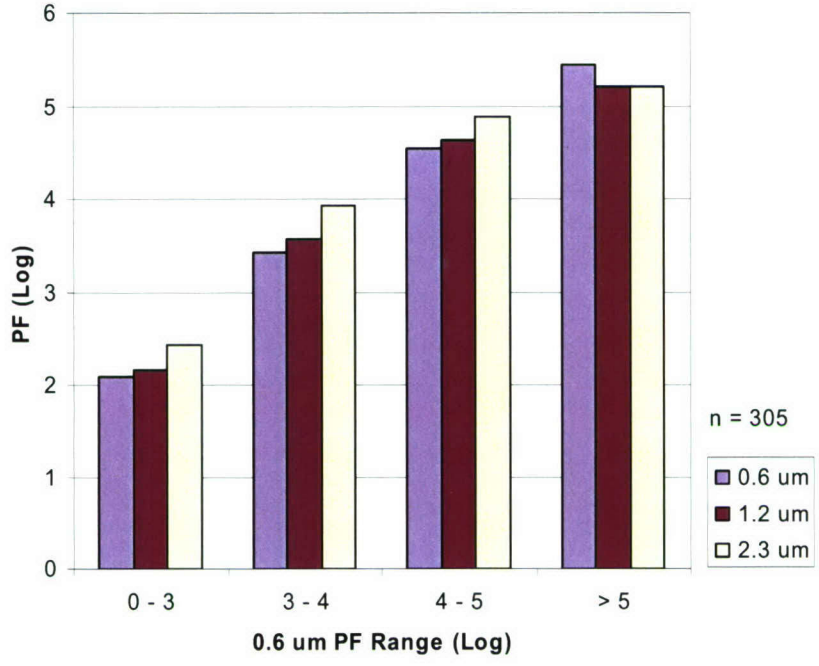

Figure 12. Averaged log PF Values within Select 0.6 μm log PF Ranges

The 1.2 and 2.3 μm log PF_{exc} values were plotted against the corresponding 0.6 μm log PF value in Figure 13. The plot is separated into three log PF ranges: 0-3, 3-4, and 4-5 (ranges 1, 2, and 3). A three-way ANOVA and post-hoc comparisons were completed on each range and on all three ranges combined. The treatments analyzed were leak type (sealed, natural, or artificial respirator leak), exercise (NB, Head S2S, Head U&D, Bend, Mimic), and particle size (0.6, 1.2, and 2.3 μm).

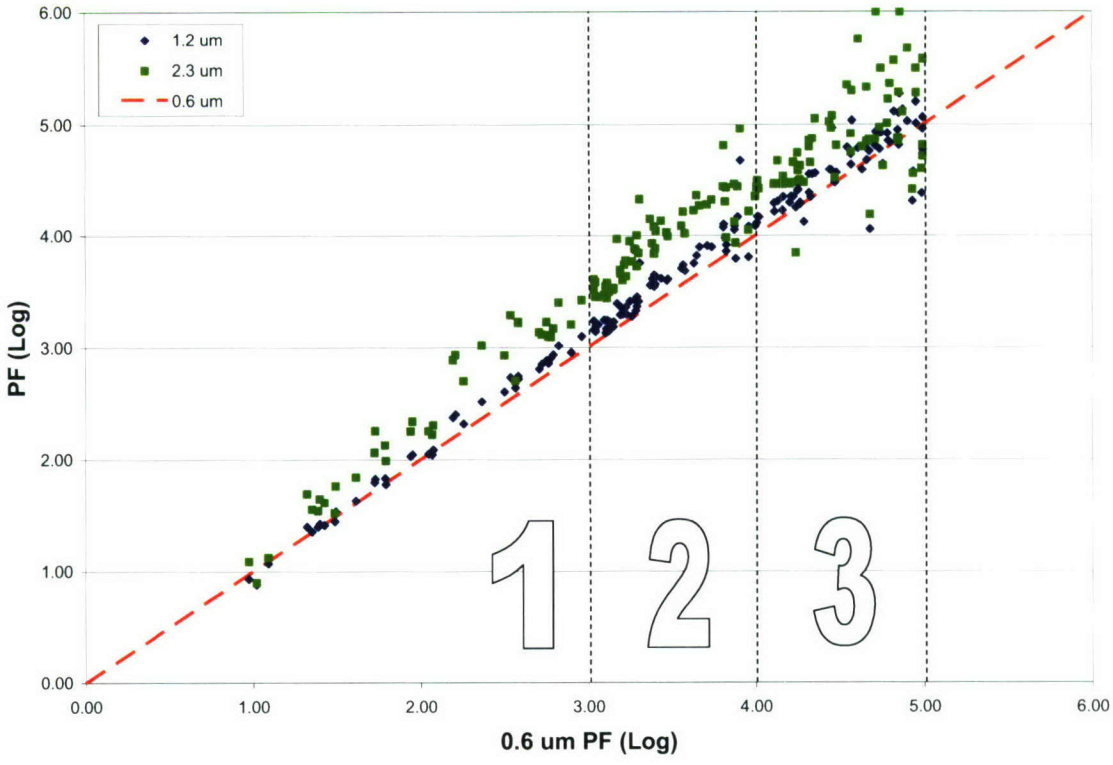

Figure 13. 1.2 and 2.3 μm log PF Values

The ANOVA analysis within each range required the use of the full trial data sets (i.e., the five exercise and three particle size PF values that made up each PF trial). To separate the trials within range 1, 2, or 3, as shown in Figure 13, the five 0.6 μm log PF_{exc} values were averaged. The 0.6 μm log PF averages were used only to separate the trial data sets. Once placed within a range, all 15 individual log PF values were used within the ANOVA analysis. Thus, it was entirely possible for several individual 0.6, 1.2, and 2.3 μm log PF_{exc} values to be either above or below the analysis range.

The ANOVA demonstrated that leak type (natural, artificial, or sealed) was significant in ranges 1 and 3 as well as all ranges combined. In range 2, only two leak types existed (natural and artificial) and values from both were scattered throughout the range. Thus, the leak type was not found to be significant within range 2. The exercise was found to be significant in all ranges (1, 2, 3, and all ranges combined). Only a few exercises in each range were statistically different, most were not. In addition, the exercise and leak type interaction was found to be significant at every range. Particle size was found to be significant in ranges 2 and 3. In range 2, the 2.3 μm log PF values were statistically different from the 0.6 μm log PF values. In range 3, the post-hoc comparison showed no statistical difference between particles, but the 0.6 and 2.3 μm particle p-value was 0.053.

The five log PF_{exe} values for each trial were averaged to find the overall trial 0.6, 1.2, and 2.3 µm log PF ($PF_{overall}$) values. The log $PF_{overall}$ results for all trials are displayed in Table 5. A two-way ANOVA was performed to analyze leak types and particle size on all trials with a log $PF_{overall}$ below 5. Leak types were significantly different while particle size was not. Post-hoc comparisons showed the sealed leak condition was significantly different than the other leak conditions.

Table 5. Trial log $PF_{overall}$ Means

log $PF_{overall}$ Means							
Trial Order	**0.6 µm**	**1.2 µm**	**2.3 µm**	**Trial Order**	**0.6 µm**	**1.2 µm**	**2.3 µm**
45	2.0	2.1	2.3	55	4.8	4.8	4.9
49	2.1	2.2	2.3	29	4.9	4.7	4.7
4	2.2	2.2	2.3	24	4.9	4.8	> 5.0
51	2.4	2.6	3.1	53	4.9	4.8	> 5.0
13	2.8	2.9	3.2	25	5.0	4.8	4.7
59	2.8	2.9	3.2	57	5.0	> 5.0	> 5.0
17	3.0	3.2	3.5	5	5.0	4.6	4.7
61	3.1	3.2	3.5	36	> 5.0	4.8	5.0
60	3.1	3.1	3.2	50[1]	> 5.0	4.9	4.9
2	3.1	3.2	3.5	28	> 5.0	5.0	4.9
23	3.1	3.2	3.5	39	> 5.0	5.0	> 5.0
7	3.2	3.3	3.6	32	> 5.0	5.0	5.0
54	3.3	3.4	3.9	3[1]	> 5.0	> 5.0	> 5.0
37	3.3	3.5	4.0	22[1]	> 5.0	> 5.0	> 5.0
26	3.4	3.6	4.0	33	> 5.0	> 5.0	> 5.0
30	3.5	3.6	3.9	27[1]	> 5.0	> 5.0	4.9
47	3.5	3.6	3.9	42	> 5.0	4.9	4.8
41	3.6	3.8	4.2	58	> 5.0	> 5.0	> 5.0
11	3.7	3.9	4.4	6	> 5.0	> 5.0	4.9
34	3.8	4.0	4.3	46	> 5.0	> 5.0	4.7
43	3.9	3.7	3.9	35[1]	> 5.0	> 5.0	5.0
9	3.9	4.1	4.3	48[1]	> 5.0	> 5.0	> 5.0
40	4.2	4.3	4.5	10[1]	> 5.0	> 5.0	5.0
14	4.2	4.4	4.5	52[1]	> 5.0	> 5.0	> 5.0
38	4.4	4.5	4.7	18	> 5.0	> 5.0	> 5.0
21	4.5	4.6	4.9	16[1]	> 5.0	> 5.0	> 5.0
31	4.6	4.7	> 5.0	12[1]	> 5.0	> 5.0	> 5.0
8	4.7	4.8	4.9	20	> 5.0	> 5.0	> 5.0
15	4.8	4.7	> 5.0	56[1]	> 5.0	> 5.0	> 5.0
19	4.8	4.9	> 5.0	44	> 5.0	> 5.0	> 5.0
1	4.8	4.9	4.9				

[1] Overall Baseline Trials

The ANOVA is a statistical method used to determine the statistical effect of nominal treatments. In the above analysis it was assumed that leak type and exercise were nominal treatments. In reality, both were more closely related to continuous variables. There are an infinite number of ways that a loose fitting respirator could seal or not seal to the subject. Thus, a better method to analyze the leakage data was by regression analysis. The leak generation method (i.e. by mask leak condition or exercise) was assumed to be unimportant. Regression analysis on particle size was performed by comparing the particle size log PF values at each exercise to a corresponding particle size log PF value. Also, only log PF data less than 5 (PF < 100,000) were used in the analysis. The 1.2 and 2.3 μm log PF values were compared to the respective 0.6 μm log PF values and fitted with a linear regression (Figure 14). Also, the 2.3 μm log PF values were compared to the 1.2 μm log PF values and fitted with a linear regression (Figure 15). It was assumed that with no protection, the PF of all three particle sizes would be 1 (log PF = 0), so the Log regression intercept was set to 0.

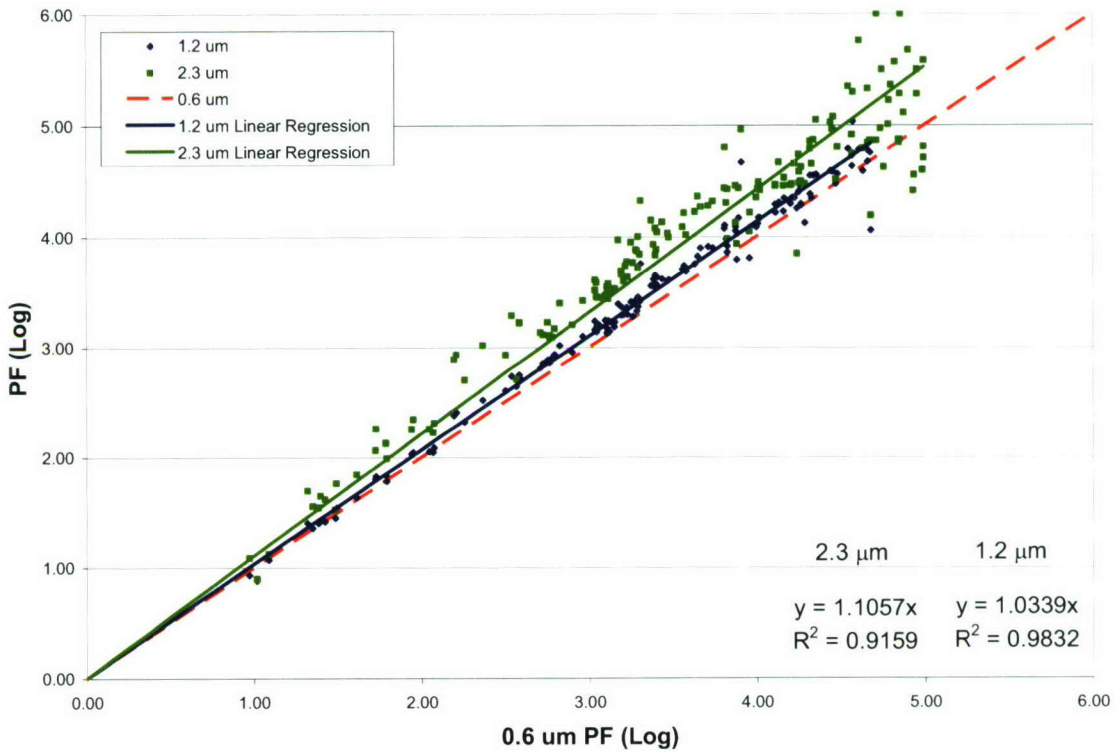

Figure 14. 1.2 and 2.3 μm log PF Regression

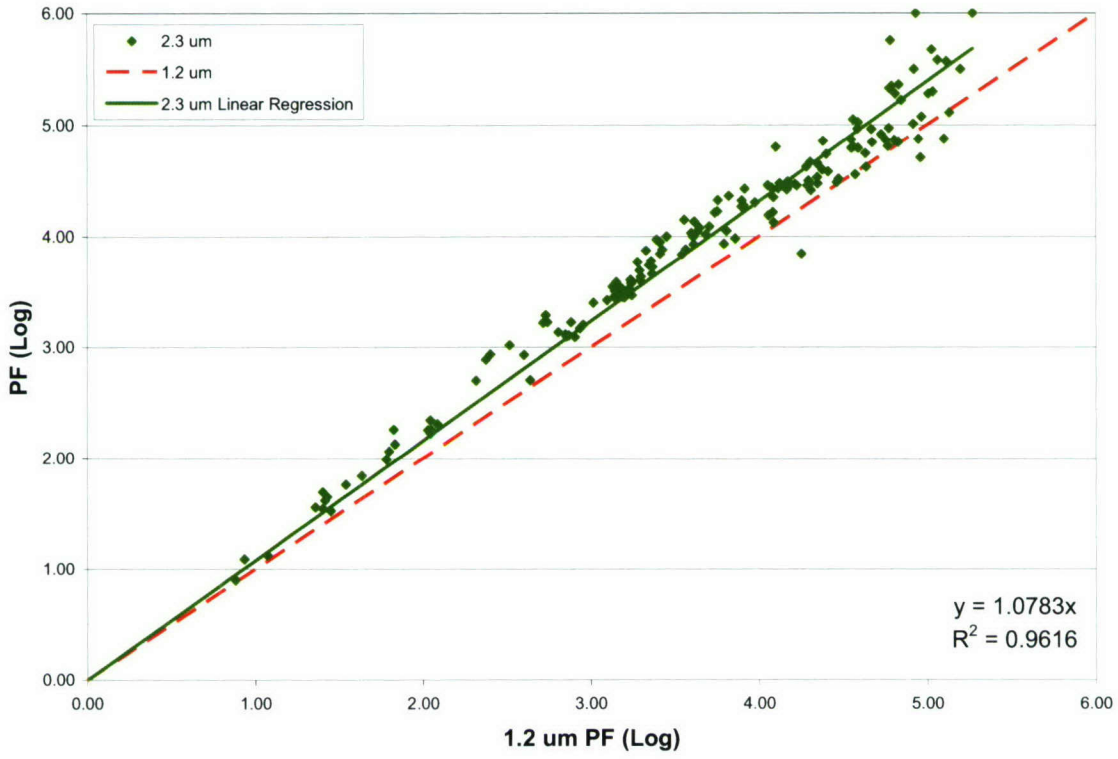

Figure 15. 2.3 μm log PF Regression

The regression slope coefficients of the 1.2 and 2.3 μm lines in Figure 14 were statistically compared to the slope coefficient of the 0.6 μm line (McCuen, 1993) (slope = 1). Also, the slope of the 2.3 μm line in Figure 15 was statistically compared to the 1.2 μm line (slope = 1). Both the 1.2 and 2.3 μm regression lines were significantly different than the 0.6 μm line, and the 2.3 μm regression line was significantly different than the 1.2 μm line. Thus, the size of the particles significantly affected the PF values. Trends within the particle size log PF data were also observed. An increase in 0.6 μm particle size to 1.2 or 2.3 μm caused the log PF values to increase by approximately 3% or 11%, respectively. Likewise, an increase in 1.2 μm particle size to 2.3 μm caused the log PF values to increase by approximately 8%.

The measured PF values are a function of challenge particle size, challenge concentration, and background concentration. PF values tended to increase as particle size increased, while challenge and background concentrations limited the maximum measured PF value. Preferably, the challenge concentrations of different sized particles would be identical and background particles would not be generated within the respirator. Unfortunately as the particle size increased, challenge and background concentrations decreased at different rates. Thus another method was devised to model measurable PF values while accounting for particle size, challenge concentration, and background concentration.

Again it was assumed that 0.6 μm log PF_{opt} values ranged from 3 to 6 (PF_{opt} ~ 1,000 to 1,000,000). These values were not calculated or measured. The linear regression equations (Figure 14) were used to calculate the 1.2 and 2.3 μm log PF_{opt} values corresponding to the 0.6 μm log PF_{opt} range. The 0.6, 1.2, and 2.3 μm average challenge concentrations from Phase 2 (C_o) were used with the PF_{opt} values to calculate the C_i values in Equation 5. The average background challenge concentrations for all three particle sizes from the background evaluation (C_b), the calculated C_i values, and the C_o values were used in Equation 3 to determine the PF_{pred} values for the three particle sizes. The log PF_{pred} results, which represent predicted measurable log PF values, are plotted against the 0.6 μm log PF_{opt} values (Figure 16).

When the 0.6 μm log PF_{opt} is less than 5, the log PF_{pred} values decrease with decreasing particle size. Hence, the measurable PF was not affected by background particles in this range. The log PF_{pred} values of the 1.2 and 2.3 μm particles fall below the log PF_{pred} values of the 0.6 μm particles at a log PF_{opt} of 5.0 (PF ~ 100,000) and 5.3 (PF ~ 200,000), respectively. At these points, the measurable PF values of the larger particles, with the lower challenge concentrations, were affected more by background particles than the measurable PF of the smaller particles. As a result, Phase 2 log PF data no greater than 4.7 (PF ~ 50,000) and 5.0 (PF ~ 100,000) were used for the 1.2 and 2.3 μm regression lines, respectively. As the 0.6 μm log PF_{opt} values increase to 6, the log PF_{pred} values for all sizes approach a maximum between 5.2 and 5.4. These maximum values were close to those predicted in Figure 9 and similar to the actual maximum PF values measured in the baseline tests. The maximum log PF_{pred} values decrease as particle size increases. This was seen in baseline tests and is attributed to background particles and lower challenge concentrations at large particle sizes.

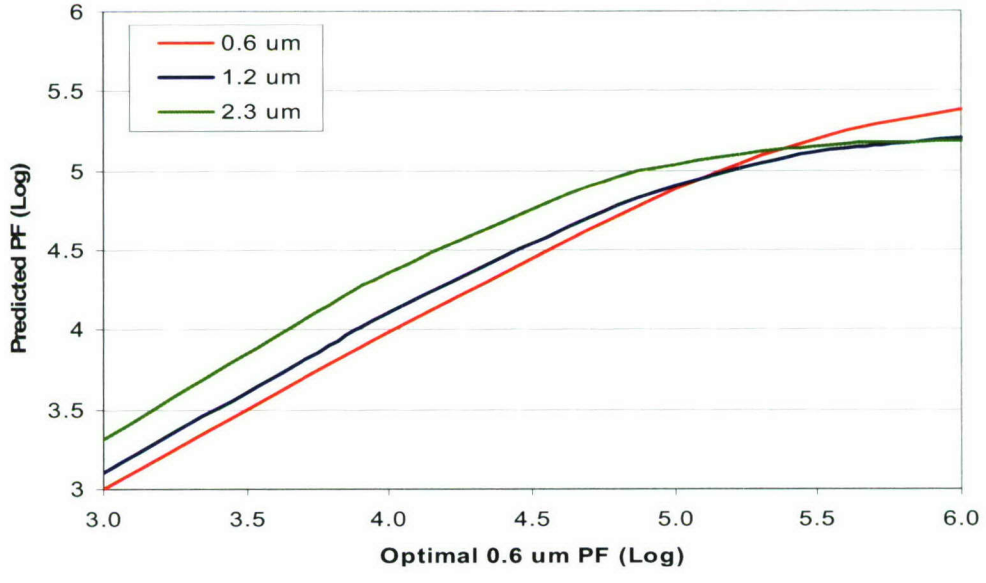

Figure 16. Predicted Measurable 1.2 and 2.3 μm log PF Values

Since predicted PF calculations used the linear regression equations, this analysis had to be completed after the linear regression analysis was completed. The results were then used to check the linear regression. The regression data range was adjusted accordingly by excluding data points used to form the regression lines in Figure 14. The excluded data points were estimated to be strongly affected by low challenge concentrations and relatively high background particles. The process was repeated until the regression was based on data estimated to be only slightly affected by low challenge concentration and background particles.

4. DISCUSSION

4.1 Phase 1–Headform Simulated Protection Factor Study

The Phase 1 simulated leakage study demonstrated that PF values as high as 1,000,000 could be measured using particle sizing/counting technology (i.e., APS) and a high monodisperse oil aerosol challenge. In addition, the results suggested that particle size can have a significant effect on the measured PF. The 5.0 μm challenge was statistically different from all four of the smaller particle sizes assessed (0.6, 1.2, 2.3, and 3.0 μm). The 1.2 and 3.0 μm challenges were the only other sizes that were statistically different from each other. Furthermore, larger particle sizes (> 1.2 μm) in most cases resulted in higher SPF values. This is a trend seen in similar studies (Hofacre and Richardson, 2001; Meyers et al., 1991). This trend is important because biological threat agents are more likely to be present on larger particulate matter (i.e., conglomerates containing more than one organism) rather than existing as submicron single-organism aerosols.

Hofacre and Richardson (2001) evaluated the effects of aerosol detection instruments and particle size on measured SPF. The study also assessed whether inert aerosols were suitable for predicting respirator performance against surrogate biological aerosols. Different particle counting instruments were used to measure SPF obtained from a full-facepiece negative-pressure respirator mounted on a headform test apparatus similar to that used in this study. The corn oil/photometer test method was found to agree well with the various particle counting methods that used monodisperse submicron polystyrene latex (PSL) spheres as test challenges. However, due to difficulties generating high concentrations of larger size particles representative of most bioaerosol threats (i.e., > 1 μm), comparisons could not be made for SPF values greater than 10,000. Within this range, SPF measured with 0.72 μm PSL spheres using an optical laser particle counter (LAS-X^{TM}, PMS) were found to be in excellent agreement with SPF measured with the 0.7 – 0.9 μm *Bacillus globigii* (Bg) spores using conventional bioassay techniques.

Meyers et al. (1991) also studied the effect of particle size on respirator leakage. In their study, SPF values (termed "apparent fit factors") were measured using challenge aerosols consisting of PSL spheres ranging from 0.36 to 2.5 μm in size. A wide range of controlled leaks were produced by inserting capillary tubes of various

diameters under the periphery of a full-facepiece mask mounted on a headform test apparatus. These researchers' findings indicated that SPF values increased as particle size increased with the effect most pronounced at the smaller capillary leak diameters.

The APS particle sizing/counting method demonstrated in Phase 1 had the ability to measure SPF values up to 1,000,000. Since particle-size specific PF values can be determined using the APS, the method provides a means for estimating respirator performance against different size biological hazards. Furthermore, previous research has shown that results from particle counting methods agree well with conventional bioassay techniques using Bg aerosol challenges (Hofacre and Richardson, 2001). Thus, the SPF method was modified only slightly for the Phase 2 human subject PF study. The main modification was the replacement of the CMAG with a Collison nebulizer. The Collison provided several advantages over the CMAG. The key advantage was that the nebulizer used compressed house air instead of nitrogen gas to generate the aerosol challenge. The nebulizer itself was also relatively inexpensive and much easier to operate and maintain than the CMAG.

4.2 Phase 2–Human Protection Factor Study

4.2.1 In-Mask Background Evaluation

Background test results confirmed the presence of subject-generated background particles within the respirator in the APS measurable size range (> 0.5 μm). A statistical analysis of the eight exercises evaluated found that the Speaking exercise created significantly more particles than the other exercises. The other seven exercises did not produce significantly different amounts of particles. In addition, only the Speaking exercise generated enough particles for the measurable PF to fall below 100,000. Based on results of previous studies (Harrison and Liang, 2005), the Speaking exercise was suspected to produce a significant amount of particles before subject PF testing began, and thus was not used. Harrison and Liang (2005) evaluated the QNFT limitations of the Portacount®. Aerosol concentrations within the respirator were measured with the Portacount® while subjects performed three exercises (normal breathing, deep breathing, and speaking) within a HEPA-filtered environment using a PAPR hood. All three exercises were shown to produce particles, but speaking created approximately ten times the concentration as normal and deep breathing. The size of the particles was not measured.

4.2.2 Baseline PF Evaluation

Since the APS PF method used in this study was unable to distinguish between challenge and background particles, it was a major concern that background particles within the respirator would create artificially low measured PF values. Thus, the average background and challenge concentrations were used to estimate the maximum measurable PF. Dividing the average challenge concentration by the average background concentration resulted in estimated maximum measurable PF values greater than 100,000. Such high PF levels were obtainable without correcting for background

contamination as evidenced by the baseline PF results. The PF_{base} levels calculated for each particle size ranged from approximately 70,000 to 800,000. However, since it was not possible to distinguish between in-mask background contamination and actual leakage, the practical upper PF measurement limit for this study was 100,000.

4.2.3 Leakage PF Evaluation

Phase 2 PF testing provided a wide range of PF values below 100,000 for comparison. As discussed in the results, PF values exceeding 100,000 were not used in the analysis. High in-mask background levels and a low challenge concentration contributed to the high variability seen in PF values greater than 100,000. Values below 100,000 were analyzed with ANOVA and regression analysis methods. The ANOVA method examined leak condition, exercise, and particle size treatments. Since leak condition and exercise were highly variable (i.e., a loose fitting mask could have produced a wide range of PF values) and repeats were virtually impossible (i.e., reproducing the same leak), the ANOVA method was used to provide only an overview of the data. Furthermore, to understand what was happening in different PF ranges, the data was broken up into three ranges before the ANOVA was preformed. The regression analysis only examined the effect of particle size on PF. All leaks, no matter how they were produced, were treated equally; thus, the high variability among leak types was not considered a factor. The regression analysis provided a better model of particle size and PF.

The ANOVA analysis indicated leak condition (artificial, natural, or sealed) significantly affected the measured PF in almost all ranges. Since the leak condition was adjusted to create a range of PF values, this was expected. Although exercise was found to be significant in all ranges, only one or two exercises (usually mimicking or bending) were significantly different than others. Most exercises tended to have no statistical difference. The leak condition-exercise interaction was found to be significant in all ranges. Since the exercises were intended to put stress on the respirator seal, this was also expected. In range 1 (PF 1 to 1,000), particle size was not significant. The leaks were relatively large so the penetration of the different particle sizes was not expected to be significantly different. In ranges 2 and 3 (PF 1,000 to 10,000 and 10,000 to 100,000, respectively), the ANOVA indicated that there were statistical differences among the particle sizes. The post-hoc test showed the 2.3 μm particles were statistically different from the 0.6 μm particles within range 2, but in range 3 the post-hoc comparison showed no statistical difference between particles. The large amount of variability observed in the PF values as they approached the method sensitivity limit most likely explains this result. An increase in the method sensitivity should result in less variability in range 3 and show that particle size is significant in this range.

The regression analysis included the statistical analysis of regression slope coefficients for each particle size. All slope coefficients were found to be statistically different from 1 (the slope coefficient of the one-to-one line). Hence, particle size significantly affected PF values. It should be noted that as a result of background particles within the respirator and a lower challenge concentration at larger particle sizes,

the regression analysis provided a conservative model. The maximum measurable PF values of the larger particles were lower than those of the smaller particles. Thus, even though the PF value of the larger particles was most likely higher, the measurable PF of the larger particles reached a point equal to that of the smaller particles. For the 1.2 and 2.3 μm particles, this was estimated to occur when the 0.6 μm particles reached a PF value of approximately 100,000. Even before the PF approached 100,000, the 1.2 and 2.3 μm PF values were affected. Hence, the slope of the resulting regression lines was presumably lower than if no in-mask background particles were present and challenge concentrations were the same. The conservative regression model still showed a statistical difference indicating particle size strongly affected PF values. Again, an increase in method sensitivity, especially at the larger particle sizes, would improve the particle size regression models.

4.3 Phase 1 and 2 Comparison

Both Phase 1 and Phase 2 particle size log PF trends were in good agreement. In both phases, an increase in the 0.6 μm particle size to 2.3 μm caused the log PF values to increase by approximately 10%. Likewise, particle size challenges above 1.2 μm led to comparable increases in log PF values. Although the log PF values increase by a relatively small percentage, the PF value is greatly affected. For example in Phase 2, a 0.6 μm particle with a log PF value of 4.0 (PF = 10,000) corresponds to a 2.3 μm particle with a log PF value of 4.4 (PF ~ 25,000). The PF value of the 2.3 μm particle is approximately 2.5 times greater than the PF value of the 0.6 μm particle. Similarly, the PF value of the 1.2 μm particle is approximately 1.3 times greater than a 10,000 PF 0.6 μm particle. Hence, a small change in a log PF value corresponds to a large change in a PF value.

5. RECOMMENDATIONS

Two approaches exist for increasing the sensitivity and reliability of the test method. The first option is to account for in-mask background particles while the second option involves increasing the particle-size challenge concentration.

To account for in-mask background particles, the respirator would first need to be expertly sized and fitted. Then, in-mask samples would have to be obtained for each exercise in a particle-free environment prior to PF testing. Since each person is different and will not produce the same levels of particles, the background test would have to be performed by all test subjects. The corrected in-mask concentration would be calculated by subtracting the pre-determined background concentration from the in-mask leakage concentration measured during PF testing. However, large errors could occur in the PF measurements if background levels should change over the course of the trial.

The second option for increasing the upper PF limit of this method is to increase the particle-size challenge concentration. For example, the polydisperse corn oil

challenge in this study contained an average 1.2 μm concentration of 2,300 particles/cc. By increasing the average challenge concentration an order of magnitude, to 23,000 particles/cc, 1.2 μm PF measurements greater than 1,000,000 can be reached. There are three ways that this can be accomplished.

The first technique would be to simply use a larger multi-jet nebulizer or adjust the air-pressure to the 6-jet Collison nebulizer used in this study to produce a greater polydisperse corn oil concentration. But, a high polydisperse concentration would flood the APS with particles, most of which are outside the target size range of interest. This could be avoided by adding an additional diluter to achieve at least a 1:1,000 dilution ratio. However, this may result in sampling biases (i.e., under- or over-sampling). In addition, the diluter may become easily clogged and require frequent cleaning.

The second technique would be to replace the nebulizer with the CMAG used in Phase 1 to provide a high monodisperse concentration of the challenge particle size of interest (e.g., 1.2 μm to simulate aerosolized anthrax spores). However, this is not the most cost-effective or practical approach since nitrogen is used as the carrier gas to generate the aerosol challenge.

The final and preferred technique would be to use a larger Collison nebulizer (e.g., 24-jet) along with a virtual impactor to separate all the smaller particles (<1.0 μm) out of the high polydisperse challenge. A virtual impactor works much like a normal impactor. Particles are separated based on inertial differences, but unlike a normal impactor, the larger particles are separated into a slower moving airflow instead of impacted on a plate. Thus, different particle sizes are separated into two air streams. The stream containing the smaller particles would be discarded, while the stream with the larger particles (> 1.0 μm) would be used as the challenge stream. By eliminating the high concentration of small particles, the APS would not need a second diluter to avoid being flooded.

6. CONCLUSIONS

An APS-based method has been developed that is capable of estimating the biological PF performance of respirators using inert oil aerosol challenges corresponding in size to different bioaerosol threat agents. In general, both simulated and actual measured PF values (obtained during subject testing) were found to increase as the size of the aerosol challenge increased. Since the APS counts particles in discrete size units ranging from 0.5 to 20 μm, it is possible to obtain several size-specific PF measurements from a single PF test by using a polydisperse aerosol challenge having sufficient concentrations of the particle sizes of interest. In subject PF test trials, the polydisperse challenge method was shown to have sufficient sensitivity to measure PF values up to 100,000. In-mask background particles generated from speaking, and to a lesser extent from the other exercises, were found to limit the maximum measurable PF. The findings suggest that it is possible to measure PF values up to one million or greater

using the APS-based method without correcting for in-mask particle background levels. The preferred approach for increasing the sensitivity of the method involves using a high polydisperse aerosol challenge (e.g., corn oil) and virtual impactor to remove the smaller particles, leaving only particles in the target size of interest (e.g., between 1 to 2 μm) in the challenge air stream. The main benefit of this technique is that it would not require the use of an additional diluter with the APS, which could bias the PF result.

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APPENDIX A

TEST SYSTEM CHARACTERIZATION

A1. INTRODUCTION

Phase 1 and 2 characterization tests were performed on the test systems described in the report. In both phases, initial data was collected to maximize the challenge concentration, determine the equilibrium time, and determine the stability of the challenge atmosphere. Some results from the shakedown characterization tests are presented below.

A2. METHODS AND RESULTS

A2.1 Phase 1 Characterization

Phase 1 shakedown tests were conducted to determine the proper operating parameters for the test setup. The CMAG screen flow, saturator flow, total nitrogen gas flow, and oil temperature were adjusted to create a specific particle size and concentration. The CMAG settings that produced the highest monodisperse particle concentrations of interest (i.e., 0.6, 1.2, 2.3, 3.0, and 5.0 μm) were experimentally determined through trial and error. Once found, the settings were recorded and reproduced during simulated leakage testing.

The time required to reach steady-state within the exposure chamber was experimentally determined. The CMAG was set to create the particle size of interest and the monodisperse aerosol allowed to fill the exposure chamber. The concentration within the chamber was monitored with the APS each minute until steady-state was reached. The 3.0 μm particle challenge shakedown test is shown in Figure A1. Testing was conducted at static flow (no simulated breathing) and three breathing flow conditions: 25, 64, and 100 L/min. These breathing flow rate conditions were chosen to simulate low, moderate, and high-intensity work rates. As the breathing rate increased, the time required to reach steady-state decreased. Steady-state was reached at approximately 25, 15, 10, and 7 minutes at no flow, 25, 64, and 100 L/min conditions, respectively.

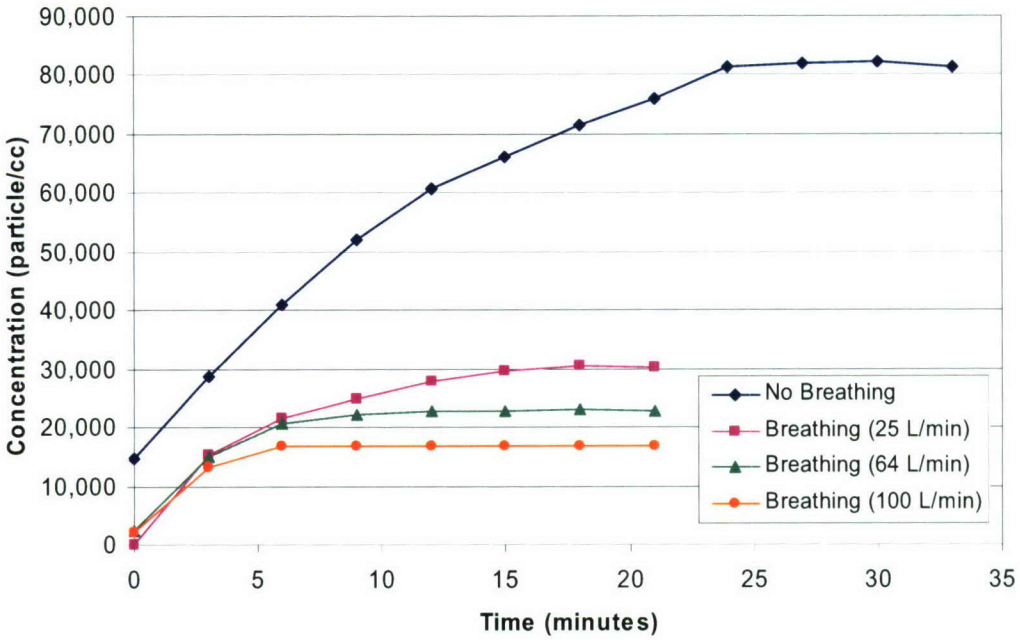

Figure A1. 3.0 μm Chamber Concentration

A 25 L/min breathing rate (25 breaths/min with a 1 L tidal volume) was chosen for Phase 1 testing because it best approximated the work rate associated with the PF test exercise regimen used in Phase 2. Figure A2 shows the maximum chamber concentration obtained for each particle size under static conditions with the breathing flow rate set at 25 L/min. As seen in Figure A2, as the particle size increased, the challenge concentration decreased. At the 25 L/min breathing rate, all the particle challenge concentrations were sufficiently high (i.e., approximately 10,000 particles/cc or greater) to permit calculation of SPF values greater than 1,000,000 after taking into account particle background levels in the test system.

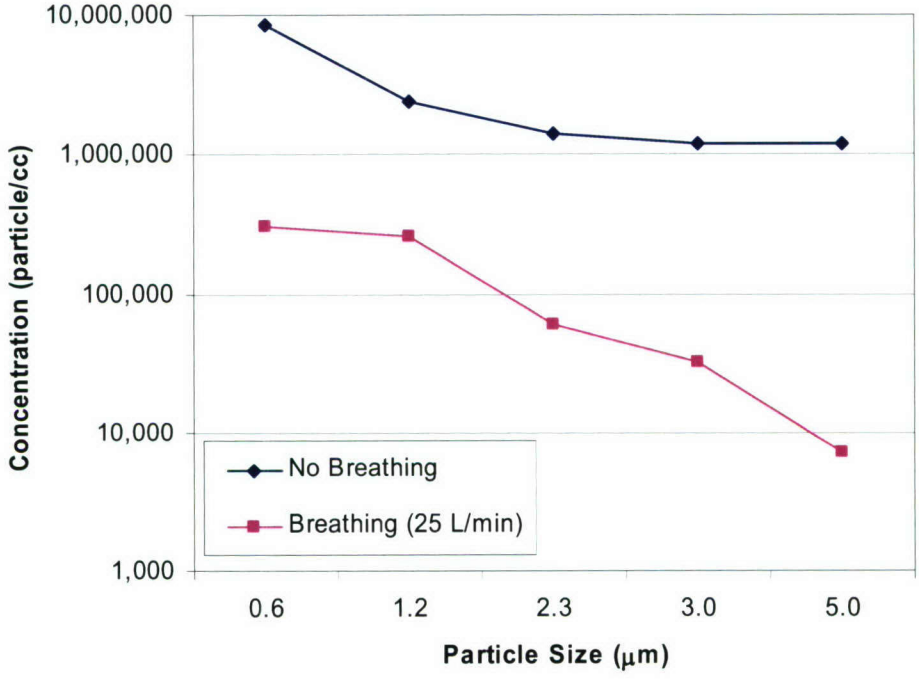

Figure A2. Chamber Concentrations at Static and 25 L/min Breathing Conditions

A2.2 Phase 2 Characterization

Initial shakedown tests were performed to determine the proper operating parameters for the Phase 2 test setup. The Phase 2 setup was much simpler than the Phase 1 setup. The only parameters considered were the vacuum blower voltage and the nebulizer flow. Both parameters were manipulated to create a polydisperse corn oil aerosol challenge distribution capable of measuring PF values as high as 1,000,000 in the 0.6, 1.2, and 2.3 µm particle size ranges without flooding the APS which has an upper count limit of approximately 1,000 particles/cc. It was assumed there were no measurable background particles inside the respirator. The in-mask sample time was set at two minutes to extend the APS detection limit to 0.0005 particles/cc. Thus, in order to measure PF values of 1,000,000 or higher, the challenge concentration for each particle size had to exceed 500 particles/cc. Through trial and error, it was determined that the proper operating conditions were met once the variable autotransformer was set to 35 volts and the Collison nebulizer to 6 L/min. It was also determined that the challenge concentration reached steady-state in approximately 20 minutes. A typical polydisperse corn oil challenge concentration distribution measured by the APS is shown in Figure A3. The average 0.6, 1.2, and 2.3 µm particle challenge concentrations were 10,300; 2,400; and 540 particles/cc, respectively.

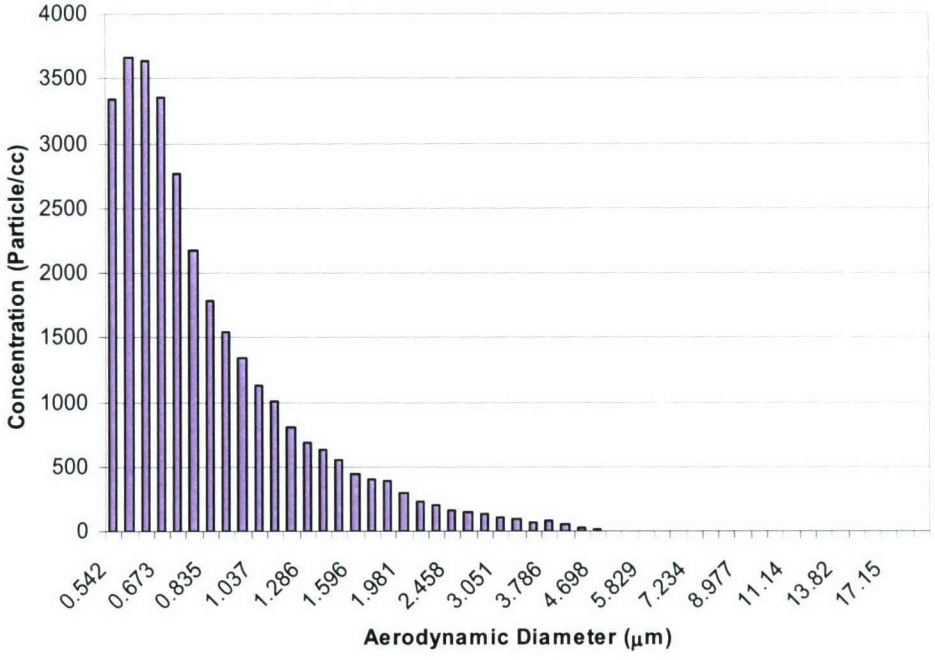

Figure A3. APS Corn-Oil Challenge Number Concentration Distribution

APPENDIX B

PHASE 2–BACKGROUND PARTICLE CHARACTERIZATION

B1. INTRODUCTION

After completing the Phase 2 PF study, the baseline PF values were not as high as expected. This was attributed to background (i.e., non-challenge) particles within the respirator. Artificially low fit factor values measured with the Portacount® fit tester were observed by Harrision and Liang (2005) due to ambient particles generated within the respirator from exhaled breath. The most likely sources of the background particles in the Phase 2 study were exhaled breath and carbon fines (i.e., filter dusting) produced by the CBRN filter canister. Thus, two shakedown tests were devised to identify and characterize the potential sources of the background particles within the respirator. An investigation was conducted to measure the background particle concentration within the respirator while human subjects completed different exercises within a HEPA-filtered atmosphere. Another investigation was conducted to check for filter dusting while the respirator and filter were mounted on a headform. A description of the test methods and results is presented within this appendix.

B2. METHODS

B2.1 In-Mask Background Evaluation

B2.1.1 Background Test System

A loose-fitting powered air-purifying respirator (PAPR) (PA20, Bullard, KY) with a modified hood was used to supply HEPA-filtered air to the test subjects. Test subjects wore a commercial 3M FR-40 air-purifying full-facepeice respirator. Extra material was sewn into the PAPR hood to allow it to comfortably surround the mask and extend past the subject's shoulders.

B2.1.2 Test Procedure

The subject donned a properly sized 3M FR-40 respirator and the seal was checked with a Portacount®. The respirator was considered sealed when the Portacount® read less than 1.2 particles/cc. Once the respirator was properly adjusted, a two-meter long 1/8-inch inner diameter (ID) Tygon® in-mask sample tube was attached to the respirator drink tube. The other end of the in-mask sample tube remained closed off. The PAPR was turned on and the hood was slipped over the subject's head and respirator. At least five minutes was allowed for the PAPR to purge the air within the hood prior to sampling.

The APS sample inlet (i.e., inner 1 L/min sensor sample port) was attached directly to the PAPR hood with a two-meter 1/8-inch ID Tygon® sample line. A one-minute APS sample was taken. Next, the APS sample inlet was attached directly to the in-mask sample line. The subject then randomly performed one of eight exercises. At least 15 seconds were allowed to pass before the APS took the sample for one minute. The subject then started another randomly selected exercise. This process was repeated until all eight exercises had been completed. The APS was again attached to the PAPR hood sample probe and a one-minute sample was taken. Once the test was completed, the subject removed the PAPR and respirator. During the sampling process, the exercise order was recorded. Additional data recorded included the length of time since the subject's last drink or meal and whether the subject smoked.

B2.1.3 Test Matrix

A total of six human subjects participated in this test. The six subjects were selected from the 11 that participated in the Phase 2 PF study. The eight exercises performed in random order were normal breathing (NB), deep breathing (DB), turning head side to side (Head S2S), moving head up and down (Head U&D), bending over (Bend), rotating jaw (Jaw R), Speaking - reciting "Rainbow Passage" (Speak), and mimicking speech – lip-synching "Rainbow Passage" (Mimic).

B2.2 Filter Dusting Evaluation

B2.2.1 Dusting Test System

A Simulated Agent Resistance Test Manikin (SMARTMAN) headform connected to a breathing machine (Computerized Breathing Simulator, FENZY, France) was used to check the CBRN filter canister for the presence of dusting. A size medium 3M FR-40 respirator was sealed to the headform. Located between the respirator and headform was an inflatable bladder connected to a pump and pressure gauge. The bladder maintained a constant seal along the periphery. The breathing machine was connected to the headform through a HEPA filter. There was no bypass; air entered the respirator through the filter canister and exited the respirator through the respirator exhaust valve. The test filter attached securely to the respirator. To prevent ambient particles from interfering with the in-mask sample measurements, a loose-fitting PAPR hood (PA20, Bullard, KY) was placed over the headform to supply HEPA-filtered air to the respirator.

B2.2.2 Test Procedure

A test was conducted on previously used filter canisters to assess the effect of breathing rate on filter dusting. The test filter was sealed to the respirator and the inflatable bladder was pressurized to 3 psi. The APS sample inlet (i.e., combined 5 L/min sensor and sheath air sample port) was directly attached to the drink tube with flexible Tygon® tubing. The PAPR hood was fit over the headform and respirator and the PAPR blower was turned on. The breathing machine was set at the initial 20 L/min breathing rate and run for five minutes to allow for background particles within the mask

to purge. Next, the APS sampled inside the respirator for one minute. After completion of the in-mask sample, the breathing machine was set to the next increased rate. Another five minutes was allowed to pass and another in-mask APS sample was taken. This was repeated for a total of four breathing flow rate conditions for each filter tested. Once the four breathing rates were tested, the breathing machine was adjusted back to 20 L/min (1 L tidal, 20 breaths/min). A screwdriver handle was used to gently tap the filter canister while the APS took a final one-minute sample. After the APS had finished sampling, the filter was removed from the mask and another used filter was attached.

A test designed to look at "initial" filter dusting (i.e., when a filter is first taken out of a package and used) was also performed. In this test, a new filter was removed from the package and mounted to the respirator. The inflatable bladder was then pressurized. The APS was set to take 30 second samples for five minutes. The breathing machine was set to 20 L/min (1 L tidal, 20 breaths/min). The APS and breathing machine were started simultaneously.

B2.2.3 Test Matrix

Canisters from 3M FR-40 masks worn during PF testing (Phase 2) and a new C2A1 canister were used in the breathing flow rate test. For the initial filter dusting test, a new 3M FR-40 and C2A1 canister were evaluated. The breathing rate conditions are shown in Table B1. The breathing rate was adjusted from the smallest to the largest during each canister test and then reset to 20 L/min for the tapping portion of the test.

Table B1. Breathing Rates Tested

Minute Volume (L/min)	**Tidal Volume (L)**	**Breathing Rate (Breath/min)**
10	0.5	20
20	1.0	20
40	1.6	25
60	2.0	30

B3. RESULTS

B3.1 Human In-Mask Background Evaluation

Results from the human subject test to characterize in-mask particulate background levels are documented in the results section of this report.

B3.2 Filter Dusting Test

The test results for the filter dusting evaluations performed under essentially particle-free challenge conditions (HEPA-filtered air) are provided in Table B2. Both the total particle concentration detected by the APS and the 1.2 μm particle size concentration are given. In addition, the estimated maximum experimental PF is given. The maximum experimental PF is estimated by dividing the average challenge concentration from Phase 2 PF testing by the average concentration of background particles ("filter dust") measured within the respirator. The table shows that as the breathing rate increased, the amount of particles within the respirator remained relatively constant. Only tapping on the canister caused a significant release of particles within the respirator. In most cases, the test filters did not generate enough carbon fines to bias the PF measurement.

Table B2. Filter Dusting under Various Breather Flow Conditions

Minute Volume	Conc. Range	Test Filters (Conc. in particles/cc)				Mean	StD	Max PF
		FR-40 Md	FR-40 Sm	FR-40 Lg	C2A1			
10 L/min	Total	0.000	0.000	0.000	0.003	0.001	0.001	>1,000,000
	1.2 μm	0.000	0.000	0.000	0.000	0.000	0.000	>1,000,000
20 L/min	Total	0.000	0.000	0.000	0.001	0.000	0.000	>1,000,000
	1.2 μm	0.000	0.000	0.000	0.000	0.000	0.000	>1,000,000
40 L/min	Total	0.002	0.001	0.001	0.000	0.001	0.001	>1,000,000
	1.2 μm	0.000	0.000	0.000	0.000	0.000	0.000	>1,000,000
60 L/min	Total	0.002	0.002	0.004	0.012	0.005	0.005	>1,000,000
	1.2 μm	0.000	0.000	0.000	0.002	0.000	0.001	>1,000,000
20 L/min Tapping	Total	0.417	2.216	0.463	0.359	0.864	0.902	32,417
	1.2 μm	0.073	0.396	0.081	0.072	0.155	0.160	15,434

The total particle concentration within the respirator with a new canister is plotted against time (Figure B1). The 3M FR-40 canister and the C2A1 canisters tested show a sharp decrease in concentration once the breathing machine is started. After approximately two minutes of breathing, the air within the respirator is virtually free of particles.

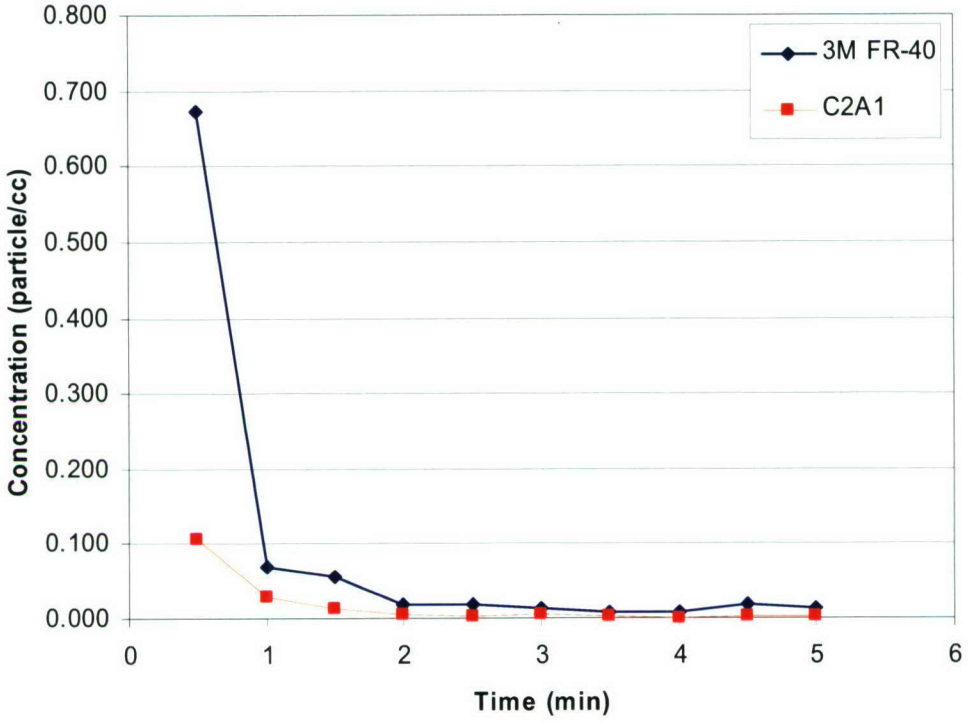

Figure B1. Respirator In-Mask Concentration for New Filters at a 20L/min Breathing Rate

B4. CONCLUSION

The human background evaluation demonstrated that background particles are present within the respirator. Also, with the exception of speaking, most exercises did not generate enough particles for the measurable PF to fall below 100,000. The filter dusting test demonstrated that particles detected during breathing were most likely not from the respirator filter. However, the relatively large release of particles after tapping the filter may indicate that exercises that jar or vibrate the filter may cause a significant amount of dusting. Thus, any future testing should include the use of HEPA filters with no organic vapor sorbent bed to eliminate any filter dusting that may occur.